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INSIGHTS INTO THE BIOLOGY OF HAPLOID MAMMALIAN CELLS

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To whom it might concern:

Dr. Óscar Fernández-Capetillo Ruiz, head of the Genomic Instability Group at the Spanish National Cancer Research Centre (CNIO) and Professor of Cancer Therapy at the Department of Medical Biochemistry and Biophysics (Karolinska Institutet), certifies that the Doctoral Thesis entitled: "**Insights into the biology of haploid mammalian cells**", developed by Dr. med. Teresa Olbrich, MSc, meets all the requirements to obtain the **PhD (Doctor of Philosophy)** degree in **Molecular Biology** and that it will be defended at the Universidad Autónoma de Madrid with the aforementioned objective. This thesis has been co-directed under my supervision and the supervision of **Dr. Sergio Ruiz-Macías**, Senior researcher in my group, and we authorize its presentation to the tribunal.

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INDEX

RESUMEN

La identificación de la función génica se ha logrado en gran medida mediante la introducción de mutaciones en el gen de interés en células y organismos. Históricamente, el uso de algunos organismos, como levaduras o insectos sociales, que solo tienen un único conjunto de cromosomas idénticos (organismos haploides) ha sido fundamental para identificar mecanismos moleculares específicos y vías de señalización esenciales en la célula mediante la realización de rastreos (*screenings*) genéticos globales. En estos rastreos, la recuperación de mutantes se basa en la selección de una característica fenotípica necesaria para identificar posteriormente los genes alterados que producen el fenotipo observado. Los organismos haploides poseen una sola copia de cada gen y, por lo tanto, su genoma proporciona un marco genético ideal para asignar una función o un fenotipo a un gen. Debido a la ausencia de un segundo alelo que pudiera compensar la ausencia del primero, todas las mutaciones son dominantes. La realización de este tipo de rastreos genéticos han sido más complicados en células de mamífero debido a sus genomas diploides, y dichos experimentos se han hecho mediante la aplicación de tecnologías propensas a efectos indirectos tales como la interferencia de ARN. Recientemente, el establecimiento de líneas celulares de mamíferos haploides ha permitido la implementación de tecnologías de rastreos similares a las que se realizan en levaduras. No obstante, el trabajo en el laboratorio con células haploides de mamífero confronta un desafío técnico importante conocido como "diploidización", y que se refiere a la pérdida de las células haploides en el cultivo, que son progresivamente reemplazadas por células diploides. Este fenómeno plantea importantes preguntas biológicas: ¿por qué el estado haploide es genéticamente inestable en células de mamífero? ¿Es posible estabilizarlo genética o químicamente? ¿Es factible generar tejido haploide en un organismo? La presente tesis trata de abordar estas preguntas.

ABSTRACT

Gene function identification has been to a large extent achieved by the disruption of the gene of interest in cells or organisms. Historically, the use of some organisms such as yeast or social insects, which only carry a single set of chromosomes (haploid organisms), has been critical to identify essential pathways and molecular mechanisms in biology through forward genetic screens**.** In these experiments, the recovery of mutants is based on the selection of a phenotype to subsequently identify the gene(s) disrupted that produce the observed phenotype. Due to the absence of a second allele, all mutations in haploid cells are dominant since no compensation by the second allele can take place. Forward genetic screenings have been more challenging in mammalian cells due to their diploid genomes, and these experiments were carried by technologies such as RNA interference that are prone to indirect effects. Recently, the development of haploid mammalian cell lines has now enabled the implementation of similar forward genetic screens as the ones performed in yeast. Yet, work with mammalian haploid cells faces a severe technical challenge known as “diploidization”, which refers to the progressive loss of haploid cells from the culture that are replaced by diploids. This phenomenon raises several important biological questions: Why is the haploid state genetically unstable in mammals? Is it possible to stabilize haploidy in mammals either genetically or chemically? Is it possible to make mammalian haploid tissue? Addressing these questions was the aim of this thesis.

ABBREVIATIONS

ACA	Anti-centromere antibodies
AD-CRE	Cre Recombinase Adenovirus
APC	Anaphase-promoting complex
BFP	Blue fluorescent protein
BSA	Bovine serum albumin
BUB1	Budding uninhibited by benzimidazoles 1
BUB3	Budding uninhibited by benzimidazoles 3
BUBR1	Budding uninhibited by benzimidazoles-related 1
CDC20	Cell division cycle 20
CDK	Cyclin dependent kinase
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
DAB	10-Deacetylbaccatin III
DAPI	4',6-diamidino-2-phenylindole
DNA	Deoxyribonucleic acid
DMSO	Dimethyl sulfoxide
EdU	5-Ethynyl 2'-deoxyuridin
EGFP	Enhanced green fluorescent protein
EGTA	Ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid
ESCs	Embryonic stem cells
FACS	Fluorescence-activated cell sorting
FAH	Fumarylacetoacetate hydrolase
FSC	Forward scatter
Q-FISH	Quantitative fluorescence in situ hybridization
H2AX	Histone H2A.X
H2B	Histone H2B
HMG-CoA	Hydroxymethyl glutaryl coenzyme A
Il2rg	Interleukin 2 Receptor Subunit Gamma
iPSCs	Induced pluripotent stem cells
KFP	Katushka fluorescent protein
KO	Knockout
LATS2	Large tumor suppressor kinase 2
LIF	Leukemia inhibitory factor
MAD1	Mitotic arrest deficient 1
MAD2	mitotic arrest deficient 2
MEFs	Mouse embryonic fibroblasts
mhaESCs	Mouse haploid embryonic stem cells
mESCs	Mouse embryonic stem cells

MMC	Mitotic checkpoint complex
MPS1	Monopolar spindle 1
MTAs	Microtubule-targeting agents
n	Number
NEBD	Nuclear envelope breakdown
NTBC	2-(2-nitro-4-trifluoro-methyl-benzoyl)-1,3 cyclohexanedione
p21	Cyclin-dependent kinase inhibitor 1
p53	Tumor suppressor protein 53
pH2AX	Phosphorylated form of histone H2A.X at serine 139
PBS	Phosphate buffer saline
PCR	Polymerase chain reaction
pH3	Phosphorylated form of histone H3 at serine 10
PI	Propidium iodide
PIPES	Piperazine-N,N′-bis(2-ethanesulfonic acid)
PFA	Paraformaldehyde
PNA	Peptide nucleic acid
RAG	Recombination-activating gene
RFP	Red fluorescent protein
SAC	Spindle assembly checkpoint
sgRNA	small guide RNA
shRNA	short hairpin RNA
SKY	Spectral karyotype
SSC	Side scatter
TOM	tdTomato fluorescent protein
UDP	Uridine 5'-diphospho
USP28	Ubiquitin carboxyl-terminal hydrolase 28
WEE1	WEE1 G2 checkpoint kinase
WT	Wild type
53BP1	Tumor suppressor p53-binding protein 1

INTRODUCTION

1. The cell cycle

The mammalian cell cycle is a highly organized and tightly regulated series of events that lead to genome duplication and cell division. It is regulated through a complex network of growth-regulatory signals and controlled by proteins surveying genome integrity to ensure the progression of the cell cycle without any DNA damage transmission to daughter cells (T. Otto & Sicinski, 2017). The cell cycle is divided into four major phases: G0/G1-S-G2-M (Figure 1) and each transition is regulated by cyclin-dependent kinases (CDKs). The activity of CDKs requires the binding of their regulatory subunits known as cyclins (Marcos Malumbres & Barbacid, 2009). Cyclins are proteins synthesized and degraded at specific time points of the cell cycle hereby tightly regulating CDK activity. CDKs driving the cell cycle during interphase are CDK2, CDK4 and CDK6, while CDK1 is the major kinase regulating entry into mitosis (Marcos Malumbres & Barbacid, 2009). In the G1 phase, cyclin D expression is induced by mitogenic signals and binds CDK4 and CDK6. Active CDK4/6-CyclinD complexes drive the expression of cyclin E, which subsequently activates CDK2 in early S-phase. In late S-phase, CDK2 is next activated by cyclin A allowing the transition from S-phase to G2/M. At this point, cyclin A activates CDK1 initiating the mitotic program. Following nuclear envelope breakdown, cyclin A is degraded facilitating the binding of cyclin B to CDK1, which becomes the major complex driving the cells through mitosis (Marcos Malumbres & Barbacid, 2009).

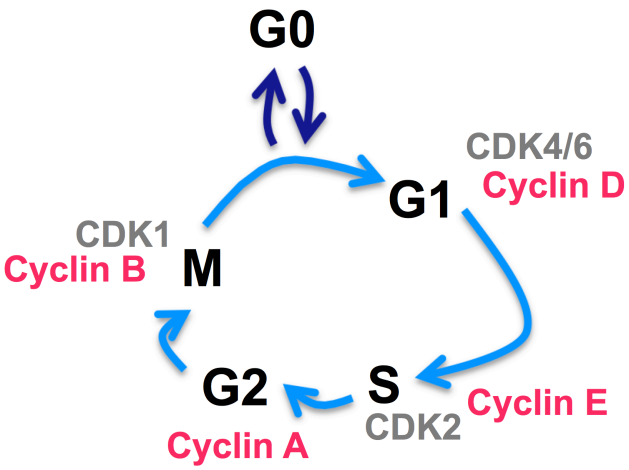


Figure 1: The cell cycle. The cell cycle consists of four consecutive, tightly regulated phases. The regulation is accomplished by cell cycle kinases that are activated at different time points by their specific subunit proteins, the cyclins.

2. An overview of mitosis

In order to divide, a cell has to replicate its genome, pack it into chromosomes and split them into two identical daughter cells. The pioneer and first descriptor of the chromosome separation associated to cell division was Walther Flemming in the late 19th century (Paweletz, 2001). He called this process “mitosis”, a greek word that literally means *thread*, reminding hereby of the shape of mitotic chromosomes observed under a microscope (Mitchison & Salmon, 2001).

We now know that mitosis is divided into five consecutive and morphologically distinct phases: prophase, prometaphase, metaphase, anaphase and telophase (Figure 2) (Musacchio & Salmon, 2007). Before entering mitosis, cells must complete an entire copy of their DNA during S-phase. Additionally, mitotic entry requires the activation of cyclin-dependent kinase-1 (CDK1), the master mitotic kinase, through its binding to cyclin B (Peters, 2006; Santamaría et al., 2007). Interestingly, how cells sense that the genome is successfully duplicated and whether or if this is directly involved in the activation of the mitotic machinery remains poorly understood (O'Connor, 2008).

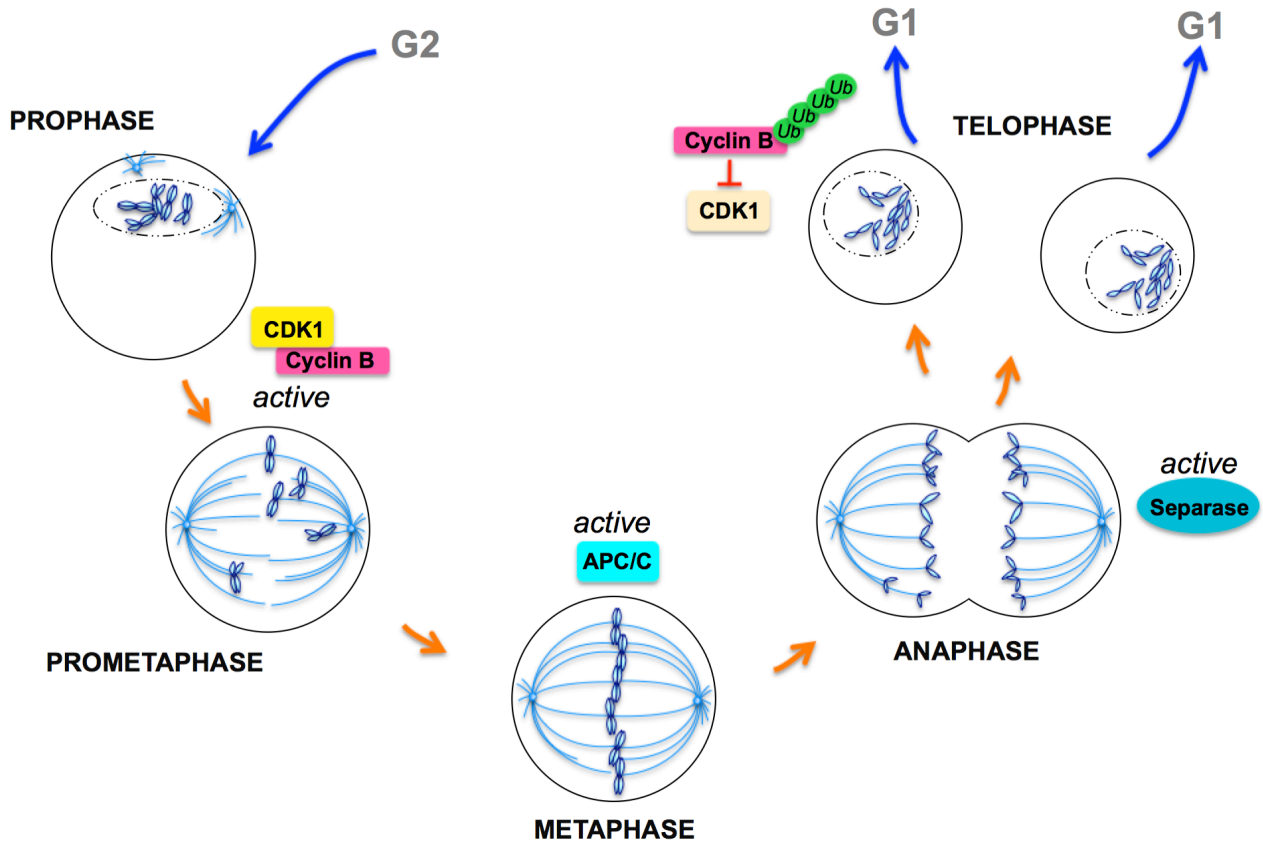


Figure 2: Overview of Mitosis. Mitosis is divided into 5 consecutive phases. Prophase is the first phase of mitosis, where chromosomes start to condensate and cyclin B levels starts to accumulate activating CDK1. Prometaphase is initiated by the nuclear envelope breakdown. In this phase chromosomes are getting attached to microtubules. Once all chromosomes are attached and properly aligned to the metaphase plate, metaphase starts followed by the activation of the anaphase-promoting complex (APC). Activated APC allows the cell to separate the sister chromatids by the enzyme separase in anaphase. In the last phase, the telophase, the chromosomes decondensate and the nucleus is reformed.

During prophase, DNA undergoes a massive process of condensation that gradually increases until metaphase. Simultaneously, the mitotic spindle, a cytoskeletal structure that will separate sister chromatids into the daughter cells, begins to form by moving the two pairs of centrioles to the opposite poles as microtubules polymerize from duplicated centrosomes

(O'Connor, 2008). Prometaphase starts with nuclear envelope breakdown. At this point, microtubules have access to chromosomes and, by rapid assembling and disassembling, engage with kinetochores, the attachment sites of chromosomes. Prometaphase ends when all chromosomes are bi-orientated, meaning that the kinetochores of all sister chromatids are connected with microtubules to the opposite poles of the spindle (O'Connor, 2008). This arrangement ensures that both daughter cells receive exactly one copy of each chromosome. At metaphase all chromosomes align in the middle of the division plane to form the metaphase plate. Metaphase is followed by anaphase, where sister chromatids are abruptly separated and moved towards spindle poles by the progressive shortening of microtubules. Once spindle poles are reached, telophase begins. During telophase the nuclear membrane is reformed and chromosomes decondensate. Finally, and after the completion of mitosis, cytokinesis initiates, leading to the partition of the cytoplasm and its content so that two daughter cells with identical genomic information are generated (O'Connor, 2008).

3. When mitosis needs extra time: The spindle assembly checkpoint

The major safeguard mechanism to ensure proper chromosome segregation in mitosis is the spindle assembly checkpoint (SAC). The SAC, active during late prophase and prometaphase, prevents anaphase onset as long as both kinetochores from each chromosome are improperly attached or non-attached to the spindle (Musacchio, 2015). Once all the kinetochores from each individual sister chromatid are properly attached to the mitotic spindle microtubules in a bi-orientated manner, the SAC becomes inactive and the cell can progress into anaphase (Figure 3) (Vitale, Galluzzi, Castedo, & Kroemer, 2011). Essential components of the SAC, including MAD1, MAD2, BUBR1, BUB1 and BUB3, were identified in the early 90s in genetic screens performed in yeast (Hoyt, Totis, & Roberts, 1991; R. Li & Murray, 1991). From a molecular point of view, current models suggest that unattached kinetochores or those lacking tension strength lead to the activation of MAD2, BUBR1 and BUB3 kinases, which cluster at the kinetochore and organize the mitotic checkpoint complex (MMC). Additional components of the SAC include MAD1, BUB1, MPS1 and Aurora-B, which catalyze the MCC formation and amplify its signal (Musacchio & Salmon, 2007). An active SAC leads to the MMC-dependent sequestration of CDC20, an activating factor of the anaphase-promoting complex (APC), which in turn is a multi-subunit E3 ubiquitin ligase complex (Vitale et al., 2011). Once the last chromosome has properly aligned with the spindle, the SAC is properly inactivated, allowing the release of CDC20, the activation of APC and the initiation of anaphase. Two APC targets are known to play central roles during mitotic progression: cyclin B and securin. Degradation of cyclin B is necessary to eliminate the high levels of CDK activity (CDK1, specifically) that raise upon entry into mitosis. In fact, cyclin B degradation

is initiated just after the SAC is inactivated and mostly depleted prior to anaphase, so that its presence is often used to measure the duration of the SAC (Clute & Pines, 1999). In addition, APC also degrades securin, an inhibitor of a protease called separase, which cleaves the cohesin rings that entrap both sister chromatids in eukaryotic chromosomes (Musacchio & Salmon, 2007). Hence, APC activation restores basal CDK levels and promotes the dissolution of sister chromatid linkage to enable their physical separation. When chromosome segregation is hampered, the persistent activation of the SAC can lead to a prolonged mitosis and, eventually, cell death, a property that has been exploited pharmacologically through the design of SAC-activating drugs as anticancer agents (see below). In summary, the SAC is a central checkpoint pathway that facilitates proper chromosome segregation during mitosis thereby limiting genomic instability.

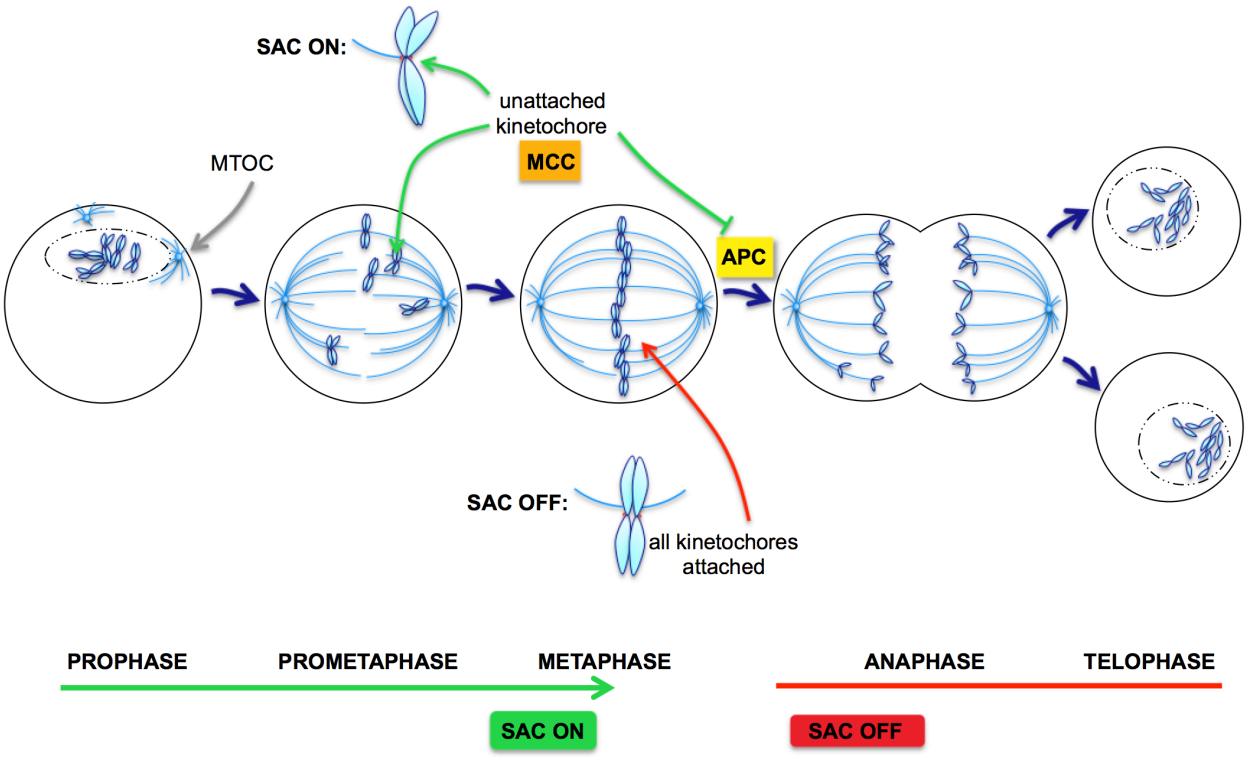


Figure 3: The spindle assembly checkpoint (SAC) is active until the metaphase-anaphase transition. The SAC is the safeguard mechanism of proper chromosome segregation in mitosis. It is active until all microtubules are properly attached to the kinetochores and thus, chromosomes are bi-orientated. Unattached kinetochores recruit proteins from the MCC, a cluster of SAC-core-proteins, leading to the inhibition of the APC complex and the anaphase onset.

4. The SAC as a target in cancer therapy

As mentioned above, targeting mitosis by chemical activation of the SAC has been exploited to attack highly proliferative cancer cells. Since cancer cells often show alterations in their ploidy such as aneuploidy or polyploidy, which complicate cell division, further impairing chromosome segregation has been used as a strategy to efficiently kill these cells. In addition,

many cancer cells present mutations in SAC components, consequently weakening checkpoint integrity (Dominguez-Brauer et al., 2015; Geert J P L Kops, Weaver, & Cleveland, 2005; Lapenna & Giordano, 2009; M. Malumbres, 2011; Pérez de Castro, de Cárcer, & Malumbres, 2007; Weaver & Cleveland, 2006). Until recently it was thought that, in contrast to what occurs in flies and yeast, the SAC was essential for cellular viability in mammals (G. J. P. L. Kops, Foltz, & Cleveland, 2004; Michel et al., 2004). Surprisingly, Raaijmakers and colleagues recently revealed that MAD1 and MAD2 are dispensable in the human haploid HAP1 cell line, both in its haploid or diploid state (Raaijmakers et al., 2018). In any case, and regardless of this specific observation, drugs that activate the SAC are a well-established therapeutic strategy.

A successful SAC-activating strategy has been the use of microtubule-targeting agents (MTAs) (Figure 4), which lead to mitotic arrest and are highly toxic for many cancer cells (Dominguez-Brauer et al., 2015). For instance, Paclitaxel and Docetaxol are well-established and clinically approved representatives of MTAs that act by inhibiting microtubule de-polymerization (Dominguez-Brauer et al., 2015). Although MTAs are indicated for the treatment of leukemia, lung, breast and ovarian cancers, their continuous application is often limited due to severe side effects, including neurotoxicity and myelosuppression.

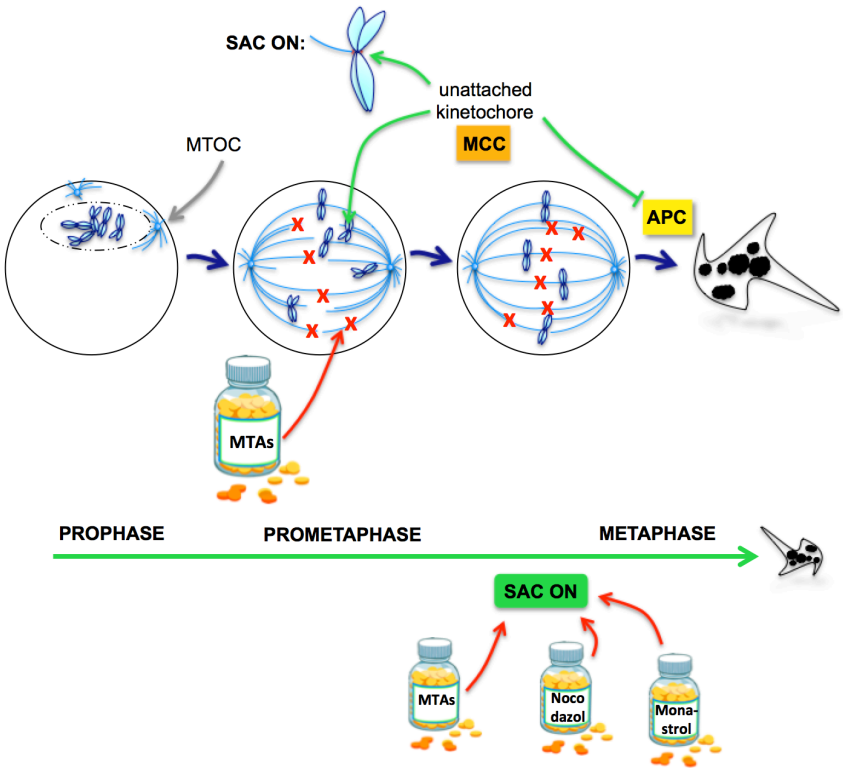


Figure 4: Chemical-induced activation of the SAC. The SAC is physiologically activated in mitosis to secure the correct and complete alignment of all chromosomes at the metaphase plate. Chemical-induced activation of the SAC can be achieved by reagents that impair the alignment of the chromosomes. MTAs, such as Paclitaxel, stabilize the microtubules imparing the cell to enter into anaphase, which subsequently will lead to its death.

Another well-established compound that activates the SAC by reversibly inhibiting microtubule polymerization is Nocodazol, a drug identified in a screen for antihelminthic compounds (De Brabander et al., 1975; Peterson & Mitchison, 2002). While this compound could not hold its anti-cancer drug potential in early clinical studies, it is a frequently used drug in biomedical research.

Finally, a different strategy to activate the SAC is through the inhibition of the mitotic kinesin Eg5 with Monastrol, a compound found in a phenotypic screening approach (Mayer et al., 1999). Inhibition of Eg5, a motor protein essential for the spindle bipolarity, induces a mono-astral phenotype and mitotic arrest (Mayer et al., 1999; Myers & Collins, 2016). Unfortunately, and despite Eg5 inhibitors reached clinical trials, the clinical data so far has also been disappointing (Myers & Collins, 2016).

Of note, it is important to bear in mind that the SAC can be activated to different degrees depending on the compound or dose used. This is due to the fact that the SAC has been shown to work as a rheostat rather than an all-or-nothing checkpoint (Collin, Nashchekina, Walker, & Pines, 2013). For instance, Nocodazol activates the SAC for longer times than Paclitaxel, although the duration of the SAC does not always correlate with the toxicity of the drugs.

5. Polyploidy in physiological and pathological conditions

Most animals possess two sets of chromosomes, thus carrying a diploid genome (Wutz, 2014). However, there are certainly examples of species with a different ploidy. For instance, there are several insects, where males are haploid and reproduce parthenogenetically whereas females are diploid and reproduce sexually – popular examples being bees, wasps or ants (Normark, 2003; S. P. Otto & Gerstein, 2008). As for polyploidy, while this is much rarer in animals than in plants, we can also find animals carrying a polyploid genome in several species of insects, fish, amphibians and reptiles (Mable, Alexandrou, & Taylor, 2011; S. P. Otto & Whitton, 2000; Van De Peer, Mizrachi, & Marchal, 2017).

In addition to ploidy differences between species, it is also possible to identify different levels of ploidy within a unique individual. For instance, polyploidy is a normal developmental pathway used to increase total organ size, when most or all of the cells in the organ are polyploid such as in Drosophila embryos (Edgar, 2006; Orr-Weaver, 2015). A similar example is found in the tomato as its size depends on the ploidy of the pericarp cells (Chevalier et al., 2014). Polyploid cells often derive from non-canonical cell cycles (endocycle and endomitosis) and/or cell fusions (Figure 5). A cell undergoing endocycles switches between G- and S-phase without entering mitosis, whereas in the endomitosis a mitotic failure leads to polyploidy (Orr-Weaver, 2015). In mammals, few organs are formed physiologically of polyploid cells, most often through

endomitosis. For instance, up to three quarters of all cardiomyocytes carry a polyploid genome (Anatskaya & Vinogradov, 2004). During early postnatal development and in response to myocardial stress, such as myocardial infarction, cardiomyocytes grow and increase in size. In most cases this hypertrophy is due to endomitosis, which has been suggested to may facilitate the contraction of the heart under stress conditions due to the bigger cell sizes (Orr-Weaver, 2015; Pandit, Westendorp, & De Bruin, 2013). Mammalian megakaryocytes are another example of polyploidy achieved by endomitosis, which are cells deriving from the hematopoietic lineage that harbor a dramatically high chromosome number. Their gigantic cell size is essential to bud off sufficient numbers of platelets from their cytoplasm (Orr-Weaver, 2015).

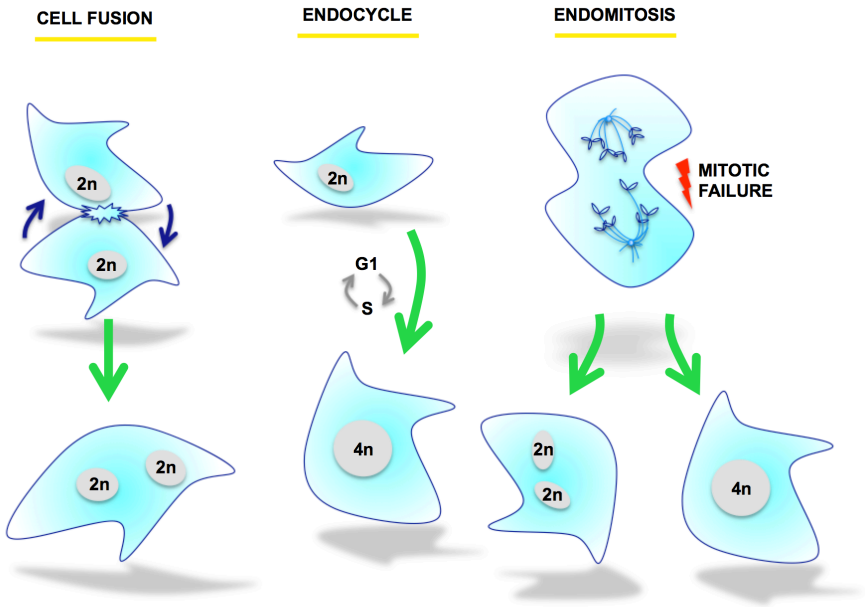


Figure 5: The road to polyploidy. Polyploid cells arise from cell fusion or aberrant cell cycles. Cell fusion leads initially to two nuclei with a final 4n DNA content. Similarly, a cell entering endocycles or endoreplication, which lack a complete mitosis after DNA replication, will also lead to a single nucleus with a 4n DNA content. Finally, endomitosis occurs due to defects in mitosis. If the defect happens before anaphase, the polyploidy cell will end up with one nuclei with 4n. Defects occurring in anaphase or later will lead to a cell with two nuclei with the same 4n DNA content.

From all organs, the liver contains the biggest amount of polyploid cells. Up to 50% of all hepatocytes in humans and 90% in rodents become progressively polyploid through endomitosis (Duncan, 2013; Gentric & Desdouets, 2014; Orr-Weaver, 2015; Zhang et al., 2018). As in other organs, the ploidy status in the liver is dynamic and may increase after surgical manipulation or in disease conditions such as in nonalcoholic fatty liver disease (Gentric et al., 2015; Tamura et al., 1992). Besides the well-known gradual accumulation of polyploid cells in the liver, it is surprising that little is known about the functional advantage, if any, of the high prevalence of polyploid hepatocytes in our organism. One hypothesis states that polyploid cells are transcriptionally more active and thus, potentially better detoxifiers (Gentric & Desdouets, 2014). Alternatively, it could also be that hepatocytes have a high resistance to DNA damage- or chromosomal instability-induced apoptosis, to avoid the premature loss of an excessive number of cells in an organ that is frequently exposed to toxins.

As mentioned above, and besides endomitosis, mammalian polyploid cells can also be generated by cell fusion. This is the case of myoblasts, involved in the formation of the skeletal muscle fibers, multinucleated osteoclasts, involved in bone metabolism and trophoblast cells, which fuse into a multinucleated syncytiotrophoblast to originate the human placenta (Pandit et al., 2013; Vignery, 2000; Xing, 2012; Zybina et al., 2002). Once again, the functional advantage of cell fusion in these tissues is currently unknown (Pandit et al., 2013).

Even if polyploidy occurs physiologically, it has also been linked to cellular transformation since it can lead to aneuploidy and subsequent genomic instability, one of the hallmarks of cancer (Hanahan & Weinberg, 2011; reviewed in (Coward & Harding, 2014)). In fact, cancer cells rarely contain exact multiples of its original genome and instead, often present complex aneuploid karyotypes (Davidson et al., 2000; Ferti, Stamouli, Panani, Raptis, & Young, 2004; Ganem, Storchova, & Pellman, 2007; Griffin et al., 2007; Pfau & Amon, 2012). Considering the high prevalence of aneuploidy in cancer, compounds that specifically target aneuploid, or genomically unstable cells in general, are in great need. However, a deep understanding of how aneuploidy perturbs the cellular machinery, and the discovery of chemical agents that can exploit these perturbations remains challenging. In this regard, a recently published chemical screen revealed that inhibition of the UDP-glucose ceramide glucosyltransferase, an enzyme involved in the sphingolipid homeostasis, is preferentially toxic for aneuploid cells *in vitro* (Tang et al., 2017). However, to what extent drugs targeting aneuploid or polyploid cells can show efficacy as an anticancer therapy remains to be tested. Finally, it should be mentioned that while some degree of aneuploidy might facilitate carcinogenesis, genomic instability is intrinsically toxic to mammalian cells and thus also in certain contexts be detrimental for cancer cells. Along these lines, a recent study has suggested that polyploidy might be tumor-suppressive in the liver (Zhang et al., 2018).

6. Haploidy in mammalian cells

Forward genetic screens have largely relied on yeast as a model organism, due to the availability of haploid individuals that facilitates these approaches. As a consequence, the isolation of animal haploid cell lines has long been sought by biomedical researchers. Initial reports on the successful isolation of haploid cell lines in frogs date back to the 1960s. Interestingly, this early work already reported that haploid cell cultures rapidly become diploid upon prolonged culture, which they suggested occurs through “diploidization"; namely the gain of a second chromosomal set. Haploid cell lines were later isolated from insects like cockroaches and flies (Debec, 1978), which faced the same limitation. The first attempt to generate primary haploid mammalian embryonic stem cells (ESCs) came in the 1980s. To do so, parthenogenesis was induced by

ethanol and, although unfertilized oocytes entered early embryonic development, all generated cell clones carried a diploid set of chromosomes (Ulrich Elling & Penninger, 2014; Kaufman, Robertson, Handyside, & Evans, 1983). Thirty years later, the establishment of the first vertebrate haploid ESC lines was finally achieved in zebrafish (Yi, Hong, & Hong, 2009). This pioneering work was followed by two other groups that succeeded in generating parthenogenetic mouse haploid ESCs (mhaESCs), modifying the original ethanol-based protocol (U Elling et al., 2011; Martin Leeb & Wutz, 2012) (Figure 6). However, once again, mhaESCs were rapidly lost in culture due to "diploidization" and thus, frequent and continuous cell sorting was essential to prevent the loss of the haploid cell population. In addition, while the aggregation of mhaESCs with diploid mESC led to chimeric mice with contribution from the haploid cells, there was no evidence of haploidy in differentiated tissues, further illustrating the rapid loss of ploidy observed in these lines (Martin Leeb & Wutz, 2012). Along these lines, subsequent work showed that differentiation of mhaESCs into embryonic cell lineages correlated with the loss of haploidy as early as the post implantation state (M. Leeb et al., 2012). Androgenetic mhaESCs have also been established, where the haploid genome derives from sperm rather than oocytes (W. Li et al., 2012; Yang et al., 2012). To date, haploid ESCs have also been generated from rat, monkey and human (W. Li et al., 2014; Sagi et al., 2016; Yang et al., 2013; Yi et al., 2009). Interestingly, human haploid ESC could be differentiated into haploid cells *in vitro* (Sagi et al., 2016), although nobody has succeeded in generating a proliferating and differentiated primary mammalian cell line to date. In addition, to what extent mammalian haploid tissue can be generated remains unknown.

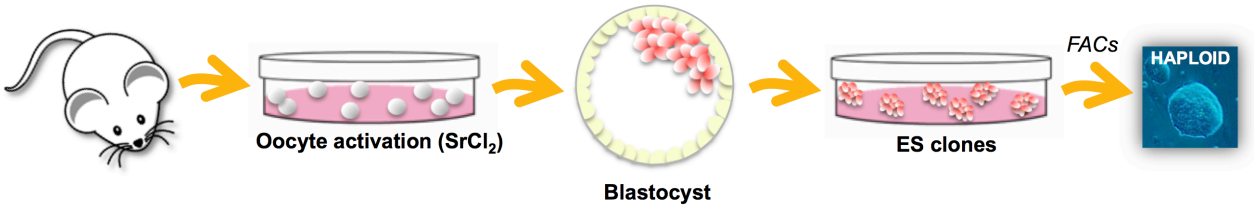


Figure 6: Derivation of parthenogenetic mhaESCs. Oocyte fertilization by the sperm sets the start point of embryonic development. However, this process can also be mimicked by the addition of strontium chloride to the oocyte, which induces blastocyst development *in vitro*. Mouse ESCs clonal lines can be isolated from the inner cell mass of these activated oocytes. Continuous cell sorting of the haploid fraction will establish over time mhaESCs.

Besides the interest of primary haploid mammalian cell lines for developmental studies, the isolation of a near-haploid cell line (KBM7) from a human patient of chronic myeloid leukemia heralded the era of haploid mammalian forward genetic screens. This human cancer cell line was initially isolated in 1995 and consisted of a mixed population of cells with different ploidy. It displayed several genomic alterations including the characteristic Philadelphia chromosome, which carries a fusion between chromosome 9 and 22 giving rise to the BCR-ABL fusion protein (Andersson et al., 1995; Ulrich Elling & Penninger, 2014). In 1999, Kotecki et al. reported the

isolation of a haploid subclone of the KBM7 cell line by serial subcloning events (Kotecki, Reddy, & Cochran, 1999). This near-haploid KBM7 subclone was haploid for all chromosomes except for the chromosome 8 (Figure 7A). Of note, the authors already suggested the unique potential of this cell line to enable forward genetic screens in mammalian cells. The first screening using KBM7 was reported ten years later and led to the identification of host factors used by pathogens for the infection (Carette et al., 2009). This pioneering work was followed by additional haploid mammalian screenings, which identified the receptors used by Ebola and Lassa viruses to infect human cells (Carette et al., 2011; Jae et al., 2014). Soon thereafter, a forward screening in KBM7 cells provided a genome-wide view of all genes that are essential for cell viability (Blomen et al., 2015; Wang et al., 2015).

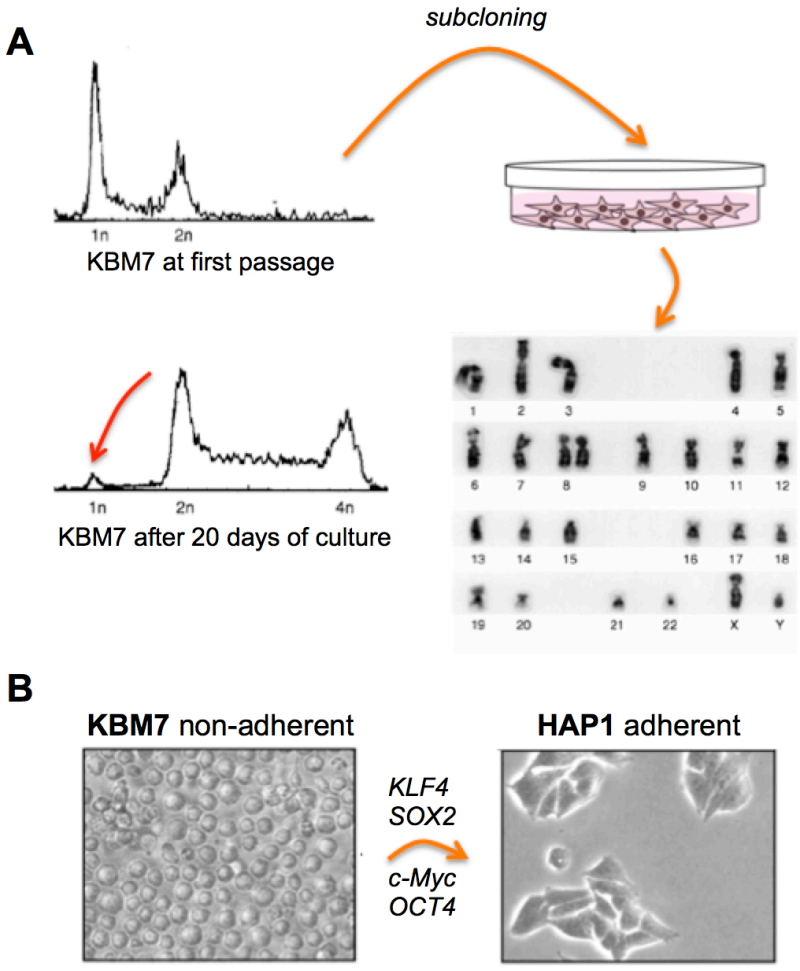


Figure 7: Isolation of a haploid human cancer cell line KBM7 subclone and generation of the HAP1 cell line. (A) Originally, it was described that the cancer cell line KBM7 suffered from a rapid diploidization in just 3 weeks of culture. Thus, subclones were generated from the heterogeneous KBM7 cell line and the P1-55 subclone, now widely used within the research community, was isolated. Karyotyping of this clone revealed a near-haploid genome (only chromosome 8 was diploid) (Modified from Kotecki, Reddy and Cochran, 1999). (B) 16 years later, Carette et al. established, in an unsuccessful attempt of inducing pluripotency in KMB7, an adherent version of the KBM7 cell line, which was named HAP1. HAP1 cells did not longer express haematopoietic markers and were haploid for all chromosomes. (Modified from Carette et al., 2011.)

While trying to generate induced pluripotent stem cells from KBM7 cells, which grow in suspension, the near-haploid adherently growing human HAP1 cell line was developed (Carette et al., 2011)(Figure 7B). Because the adherent growth facilitates their use *in vitro*, HAP1 cells became the cell line of choice and have been extensively used for forward genetic screenings. Recent examples include the discovery of regulators of tubulin detyrosination (Nieuwenhuis et al.,

2017) or the identification of the essential enzyme PLA2G16 as a picornavirus host factor (Staring et al., 2017). In fact, due to the enormous potential of this cell line and the surrounding excitement, the company *Haplogen*, which generates and provides individual engineered HAP1 cell lines for research purposes, was founded. Unfortunately, and despite de usefulness of these cells, the haploid state of KBM7 and HAP1 is also unstable.

The availability of mammalian haploid cell lines has revolutionized forward genetic screenings in mammalian cells and provided a unique tool to perform these studies. However, researchers working with haploid cells invariably face the problem of "diploidization". This is particularly acute in haploid ESC lines. In fact, the establishment and maintenance of mhaESCs requires continuous cell sorting in order to maintain a significant proportion of haploid cells (U Elling et al., 2011; Martin Leeb & Wutz, 2012). In any case, this phenomenon is not unique to mhaESCs and has been described in cell lines including frog, drosophila, rat and human ESC lines as well as KBM7 and HAP1 cell lines (Carette et al., 2011; Debec, 1984; Freed, 1962; Freed & Mezger-Freed, 1970; Kotecki et al., 1999; W. Li et al., 2014; Sagi et al., 2016; Yilmaz, Peretz, Sagi, & Benvenisty, 2016). Given the generality of this phenomenon, and the limitations it impinges to the use of mammalian haploid cells for biomedical research, we here sought to identify its causes, and tried to discover genetic and chemical ways to stabilize the haploid state in mammalian cells.

OBJECTIVES

1. To identify the molecular mechanism behind the progressive loss of haploid mammalian cells in culture.

2. To generate differentiated haploid mouse tissue *in vivo*.

3. To identify chemicals capable of stabilizing the haploid state in mammalian cells.

MATERIAL AND METHODS

Cell culture

The HAP1 cell line was a kind gift of Dr. Thijn Brummelkamp, NKI, Amsterdam and grown in IMDM (Invitrogen) supplemented with 15% FBS (Sigma), 1% P/S and 1% Glutamine (Carette et al., 2011). Mouse embryonic fibroblasts (MEFs) were obtained from 13.5 dpc embryos by standard methods and cultured in DMEM (Invitrogen), 15% FBS and 0.1 mM non-essential amino acids in low-oxygen conditions. To generate feeder layers, MEFs at early passages were growth arrested by ionizing irradiation (IR) with 80 Gy for 30 min. MEFs were immortalized by lentiviral expression of the SV40-T121 antigen following standard procedures. DLD1 cell lines were a kind gift of Erich Nigg (University of Basel, Switzerland) and Spiros Linardopouplos (ICR, London, UK) and cultured in RPMI, 10% FBS and 1% P/S. HEK293T (American Type Culture Collection) cells were grown in DMEM (Invitrogen), 10% FBS and 1% P/S. Mouse induced pluripotent stem (iPSCs) cell lines were a kind gift of Sagrario Ortega (Mouse Transgenic Unit, CNIO, Madrid) and cultured in DMEM, 20% KnockOut Serum Replacer (KSR), 0.1 mM non-essential amino acids, 1% P/S, 1% Glutamine plus 1000 U/ml LIF. The CyclinB$^{1-164}$-mCherry expressing U2OS cell line was a kind gift of Dr. Marcos Malumbres (Cell Division Cancer, CNIO, Madrid) and cultured in DMEM (Invitrogen), 10% FBS and 1% P/S. When indicated the following compounds were used to treat the cells: Doxorubicin (D1515, Sigma), DAB (Deacetylbaccatin III, S2409, Selleckchem), Paclitaxel (T7402, Sigma), Nocodazole (M1404, Sigma). For clonogenic assays, 500 HAP1 cells were seeded per well on six-well plates. After 5 days, cells were fixed, stained with methylene blue at 0.33% (w/v) in methanol followed by washes in water and air-dried. Colony size was measured and analyzed with the Image J software. *P53*-deficient HAP1 cells were generated by transfecting the CRISPR-based plasmids using the Amaxa® Nucleofector kit (Reactive L, X-001 program). A total of 5x10^5 cells were transfected with 10 µg of pX330-sgRNA-p53 plasmid.

Derivation and culture of mhaESCs

mhaESCs were generated following a reported protocol (Martin Leeb & Wutz, 2012) but using oocytes isolated from *p53*$^{+/-}$ or *p53*$^{+/-}$*; Kathuska (KFP)*$^{T/+}$ female mice. mhaESCs were cultured on a feeder layer of MEFs in gelatin-coated plates at 37°C in N2B27-based medium plus 1000 U/ml LIF, PD0325901 (1µM), CHIR99021 (3 µM) and supplemented with 15% knockout serum replacement (Invitrogen), 0.1 mM non-essential amino acids and 0.35% BSA fraction V. DNA was extracted from the cell clones using standard procedure.

Plasmids

The plasmids pX330-U6-Chimeric_BB-CBh-hSpCas9 (Addgene, 42230) and pLentiCRISPR-V2 (Addgene, 52961) were used in cells for gene editing. The sequences of the

sgRNAs used were designed and cloned as described by using the MIT CRISPR design tool (http://www.genome-engineering.org/crispr/).

Oligonucleotides	
pX330-CRISPR-p53-F	CACCGTGAAGCTCCCAGAATGCCAG
pX330-CRISPR-p53-R:	AAACCTGGCATTCTGGAGCTTCAC
pX330-CRISPR-V2-P21[1]-F	CACCGACTGGAGGGTGACTTCGCCT
pX330-CRISPR-V2-P21[1]-R	AAACAGGCGAAGTCACCCTCCAGTC
pX330-CRISPR-V2-P21[2]-F	CACCGAGTCGAAGTTCCATCGCTCA
pX330-CRISPR-V2-P21[2]-R	AAACTGAGCGATGGAACTTCGACTC
pX330-CRISPR-V2-USP28[1]-F	CACCGACCCCAATCCCAATGACTGG
pX330-CRISPR-V2-USP28[1]-R	AAACCCAGTCATTGGGATTGGGGTC
pX330-CRISPR-V2-USP28[2]-F	CACCGCTCCAGTAGACTCAAAGCAA
pX330-CRISPR-V2-USP28[2]-R	AAACTTGCTTTGAGTCTCTGGAGC

Only sgRNAs with the higher scores and lower probabilities of generating off-target effects were selected. pLenti-H2B-EGFP and pLenti-H2B-RFP were a kind gift from Dr. Marcos Malumbres (Cell Division and Cancer Group, CNIO, Madrid). The lentiviral plasmids pLVTHM (Addgene, 12247), pHIV-tdTomato (Addgene, 21374), pKLV-U6gRNA(BbsI)-PGKpuro2ABFP (Addgene, 50946) were used to express EGFP, tdTomato or BFP fluorescent proteins after infection, respectively. Down-regulation of human P53 was generated using the plasmid pLVTHM-shRNAp53 as earlier described (Kawamura et al., 2009). All newly generated constructs were sequenced entirely to rule out the presence of mutations.

Lentiviral production

Lentiviral vectors were individually co-transfected with 3rd generation packaging vectors in HEK293T cells using Lipofectamine 2000 (Invitrogen) to generate viral supernatants as described (Ruiz et al., 2011).

Conditional *P53* deletion

To generate cells with conditional expression of mouse *P53*, pSico (Addgene, 11578) and pSico-*P53* (Addgene, 12089) plasmids were used. Cells infected with pSico-*P53* express shRNAs targeting *P53* from a Cre-dependent excisable gene cassette. To express Cre and restore P53 expression, cells were infected with adenoviruses expressing Cre (Ad5CMVCre-EGFP; VVC-U of

Iowa-1174). In brief, 10^5 mhaESCs were infected in suspension with lentiviruses encoding for pSico (Addgene, 11578) or pSico-*P53*. Five days after, we sorted haploid cells based on DNA content and EGFP expression. Subsequently, we infected a total number of $2x10^5$ haploid-sorted ESCs with Ad-Cre viruses in suspension. After infection, we grew the cells on feeder layers to evaluate DNA content and P53 expression levels by WB and flow cytometry at different time points, respectively.

Flow Activated Cell Sorting (FACs)

HAP1, immortalized MEFs and mhaESCs were trypsinized followed by Hoechst staining (10 µg/ml Hoechst 33342, Thermo Fisher Scientific) for 30 minutes at 37°C. Sorting for specific haploid, diploid or tetraploid cell populations was based on FSC/SSC and/or expression of tdTomato, EGFP or BFP on a BD Influx™ cell sorter (BD Biosciences). In brief, cells were trypsinized and a small sample was stained with Hoechst and analyzed on the cell sorter to identify the population of interest in the FSC/SSC dot plot through back gating. This was possible as haploid cells were considerably smaller in size and tetraploid cells were considerably bigger in size. Subsequently, HAP1 cells were then sorted for DNA content but based on the FSC/SSC parameters. In a mix culture of haploid/diploid cells, haploids were sorted based on the G1 haploid (1n) and diploids on the G2/M diploid (4n) peaks, respectively. In a mix culture of diploid/tetraploid cells, diploids were sorted based on the G1 diploid (2n) and G2/M tetraploid (8n) peak, respectively. Single cell sorting was conducted by collecting one cell per well on 96-well plates. The purity of the sorted cells was checked after each sort by flow cytometry analysis.

Flow cytometry

We routinely analyzed the cell cycle profiles by flow cytometry. Briefly, trypsinized cells were stained with 10 µg/ml Hoechst 33342 for 30 minutes at 37°C or fixed with 70 % ethanol overnight at -20°C and subsequently stained with propidium iodide (PI) following a standard protocol. We recorded the analytic flow profiles of the DNA content on a BD Fortessa™ (BD Biosciences). At the same time or independently, we monitored and recorded the tdTomato$^+$, EGFP$^+$ and BFP$^+$ HAP1, DLD1 or immortalized MEF populations. Data was processed with the Flow Jo 10™ software.

High-Throughput Chemical Screening

HAP1 haploid cells expressing tdTomato$^+$ and HAP1 diploid cells expressing EGFP$^+$ were mixed in a ratio 4:1 and a total of 2500 cells were seeded per 96-well plate. Cells were treated individually in the wells with a FDA approved drug-screening library (Z145127, Selleckchem) containing 987

compounds (Table 2). Fresh compounds were added twice per week, cells passaged once per week and the screen was carried out in duplicates. After 3 weeks of treatment, cells were trypsinized, incubated with DAPI and analyzed the expression of tdTomato and EGFP by high throughput flow cytometry (BD FACS Canto II™, BD Biosciences). Data was processed with the Flow Jo 10™ software.

Western blot

Cell pellets were lysed in 50mM Tris, 150mM NaCl, 1% TritonX-100 or in 50mM Tris pH 7.9/8M Urea/1%Chaps followed by 30 min of incubation time shaking at 4°C. 15-25 µg of supernatants were run on precast gels and transferred for protein detection by using the following antibodies: p53 (1:1000, Cell Signaling, #2524); p21 (1:500, Cell Signaling, #2947); USP28 (1:1000, Abcam, #ab110744), CDK2 (1:2000; sc-163, Santa Cruz) and Tubulin (1:50000; Sigma, #T9026).

Immunofluorescence

Cells were fixed with 4% PFA followed by permeabilization with 0.1% Triton-X100. Cells stained for detecting MAD2 expression were pre-extracted for 60 seconds in PEM buffer (100 mM PIPES, 10 mM EGTA, 1 mM MgCl and 0.1 % Triton X-100). Antibodies against 53BP1 (1:3500, Novus 100-304A2), γH2AX (1:1000, Millipore 05-636), γTubulin (1:1000, Sigma #T6557), αTubulin (1:1000, Sigma #T9026), pH3 (1:100, Millipore 06-570), MAD2 (1:100, Bethyl Laboratories A300-301A) anti-centromeric antibody (ACA, 1:500, kind gift of Marco Malumbres, Cell Division and Cancer Group, CNIO, Madrid) were used. Images were aquired using a Leica TCS-SP5 equipped with a 0.7 NA 20x oil or 1.4 NA 63x oil (HCX plan Apo CS) objective and LAS AF 2.6 software. To generate the overlays of the spindles shown in Figure 18A, 25 metaphase spindles for each clone were acquired with the 2 poles visible in the same plane, followed by spindle centering leading to the same position for each spindle. A 400x400 pxl cropped area was generated for each channel to create a stack of all the captures from the different conditions. Subsequently, the average projection of the 25 metaphases for each condition and channel was created and the overlay of the 3 channels is displayed. HCS CellMask (Thermo Fisher Scientific) used to calculate mitotic cell size was obtained from Thermo Fisher. Images were analyzed and processed using imaging Leica Microsystems, Fiji softwares or Definiens Developer XD™ software.

High-throughput microscopy

High-throughput microscopy analyses were carried out as previously described (Toledo, Murga, Gutierrez-Martinez, Soria, & Fernandez-Capetillo, 2008). Briefly, a total of 25000 ESCs per

well or 10000 HAP1 cells were seeded on µClear® bottom 96-well plates (Greiner Bio-One) pre-treated with gelatin 0.1%. γH2AX (1:1000, Millipore, 05-636), H2AX (1:1500, Abcam ab11175) and pH3 (1:100, Millipore 06-570) immunofluorescence was performed as described above. Images from each well were automatically captured by an Opera High-Content Screening System (Perkin Elmer) at non-saturating settings. Images were segmented using the DAPI staining to generate masks matching cell nuclei from which the total nuclei intensity or number of foci was calculated and data was represented with the Prism software (GraphPad Software). For EdU incorporation analyses, EdU was added to the media at a final concentration of 20 µM for 30 minutes. EdU incorporation was detected using the Click-iT^{TM} EdU Alexa Fluor® imaging kit (Invitrogen/Molecular probes).

Cell viability assays

Cells were seeded at 25000 cells per well in a 96-tissue culture plate coated with gelatin 0.1% and treated with the indicated compound and indicated time. Cell viability was measured using a luminescent system (CellTiter-Glo, Promega) according to the manufacturer's protocol and the viability is plotted as percentage of viability compared to untreated control.

Metaphase spreads

HAP1 cells were arrested at mitosis with overnight treatment with 100 ng/ml Colcemide (GIBCO/BRL). Cells were then collected, incubated in a hypotonic buffer (0.075 mM KCl) for 15 min at 37°C and fixed with Carnoy's buffer (methanol-glacial acetic acid, 3:1). To obtain metaphase spreads, cells were dropped on slides and stained with Giemsa solution. Images from metaphases were captured and a minimum of 19 metaphases were analyzed.

Spectral karyotyping (SKY) analysis

For molecular cytogenetic analysis, ESCs were exposed to colchicine (0.5 µg/ml) for 4 h at 37ºC, and harvested routinely. Metaphases were prepared following a conventional cytogenetic protocol for methanol-acetic acid (3:1)-fixed cells. Slides were prepared from the fixed material and hybridized using the SKY method, according to the manufacturer's protocol (Applied Spectral Imaging, Migdal Ha'Emek, Israel). Images were acquired with an SD300 Spectra Cube (Applied Spectral Imaging) mounted on a Zeiss Axioplan microscope using a custom-designed optical filter, SKY-1 (Chroma Technology, Brattleboro, VT). At least 15 different metaphases were captured and analyzed in several single-cell sorted ESC clones.

Live cell imaging

To evaluate mitosis entry and duration, ESCs or HAP1 cells were infected with lentiviruses encoding the histone H2B-EGFP or H2B-RFP and seeded on 8 wells µ-Slide (Ibidi, 80826) pre-treated with gelatin 0.1%. The day after, ESCs were imaged every 15 minutes for a total of 24h with a 20x objective in a Leica DMI 6000 B system. To quantify SAC-dependent time, HAP1 cells were treated with the indicated compounds and imaged every 4 minutes for a total of 16h with a 20x objective in a Leica DMI 6000 B system. Overall mitotic duration was scored based on visual chromatin condensation/de-condensation. For a more careful evaluation of mitosis, SAC-dependent time was defined as the time from NEBD until the observation of a metaphase plate and SAC-independent time from metaphase plate to chromosome decondensation. At least, 30 cells were followed to evaluate the time spent in mitosis and interphase as well as cell fate for each individual cell.

To evaluate the cyclin B degradation, U2OS cells expressing a CyclinB-mCherry fusion protein were seeded on 8 wells µ-Slide (Ibidi, 80826). The following day, cells were treated with the indicated compounds and imaged every 4 minutes for a total of 16h in a Leica DMI 6000 B system. Cyclin B degradation was evaluated in cells from the NEBD, with the maximum level of cyclin B expression, until the start of anaphase, where mCherry signal was lost entirely.

Mice

P53$^{+/-}$ female mice (Jacks et al., 1994) were used to generate P53-deficient mhaESCs. *P53*$^{-/-}$ male mice (Jacks et al., 1994) were crossed with *KFP*$^{T/+}$ female mice (Diéguez-Hurtado et al., 2011) to obtain *P53*$^{+/-}$; *KFP*$^{T/+}$ female mice to generate *P53*$^{-}$; *KFP*^{T} and mhaESCs. *FAH*$^{-/-}$; *Rag2*$^{+/+}$; *Il2rg*$^{+/+}$ female mice (Azuma et al., 2007) were used to generate *FAH*$^{-/-}$ blastocysts. *FAH*$^{-/-}$ animals were maintained with 2-(2-nitro-4-trifluoro-methyl-benzoyl)-1,3 cyclohexanedione (NTBC, CuRX^{TM} Nitisinone, Yecuris Corporation; #104206-65-7)-containing drinking water at a concentration of 10 mg/l. In collaboration with the Transgenic Mice Unit of the CNIO, newly generated *P53*$^{-}$; *KFP*^{T} mhaESCs were microinjected into *FAH*$^{-/-}$ blastocysts and transplanted into female foster mice. NTBC was eliminated of the drinking water at E10.5.

Genotyping of the DNA from mouse-tails was performed following standard procedure. The following primers were used: FAH-F 5' TTGCCTCTGAACATAATGCCAAC 3'; FAH-R^{WT} 5' TGAGAGGAGGGTACTGGCAGCTAC 3'; FAH-R^{MUT} 5' GGATTGGGAAGACAATAGCAGGC 3'; Il2rg-F 5' CTGCTCAGAATGCCTCCAATTCC 3'; Il2rg-R^{WT} 5' ACCGTTCACTGTAGTCTGGCTGC 3'; Il2rg-R^{MUT} 5' GGTCGCTCGGTGTTCGAGGCCAC 3'; Rag2-F^{WT} 5' GGGAGGACACTCACT TGCCAGTA 3'; Rag2-F^{MUT} 5' CGGCCGGAGAACCTGCGTGCAA 3'; Rag2-R 5' AGTCAGGAG TCTCCATCTCACTGA 3'; KFP-F 5' AACGACCACCACTTCAAGTGC 3'; KFP-R 5'

TAGCCAGAAGTCAGATGCTCAAGG 3'; p53 F1 5' TGGTTTGTGCGTCTTAGAGACAGT 3'; p53-F2 5' CCAGCTCATTCCTCCCACTCA 3'; p53-R 5' AAGGATAGGTCGGCGGTTCAT 3'.

Embryos or newborn pups were fixed in formalin, embedded in paraffin/formalin blocks and serial sections of 5 μM were cut. H&E staining was performed following a standard protocol. For immunohistochemistry analyses, the slides were treated with citrate buffer for antigen retrieval and stained with an antibody against Turbo-RFP (Katushka, Evrogen #AB233) following standard procedures. Immunohistochemistry slides were scanned and digitalized with a MIRAX system from Zeiss. Mouse work was performed in accordance with the Guidelines for Humane Endpoints for Animals Used in Biomedical Research and under the supervision of the Ethics Committee for Animal Research of the "Instituto de Salud Carlos III".

Immunofluorescence followed by FISH (Immuno-FISH) on embryo sections

Immunofluorescence was performed on deparaffinized embryo sections processed with 10 mM sodium citrate (pH 6.5) cooked under pressure for 2 min for antigen retrieval. Tissues were permeabilized with 0.5% Triton X-100 in PBS for 1.5 h (3 x 30 min) at room temperature. Samples were blocked with PBS with 5% BSA for 3h and incubated overnight at 4 °C with an antibody against Turbo-RFP (Katushka, Evrogen #AB233) diluted 1:100 in PBS 5% BSA. Slides were washed three times for 15 min with 0.1% Tween 20 in PBS and incubated with an Alexa Fluor 488 anti-rabbit (ThermoFisher Scientific A11008) secondary antibody for 1h at room temperature. Samples were washed three times for 15 min with PBS with 0.1% Triton X-100 and then fixed for 20 min in 4% paraformaldehyde in PBS. Quantitative centromere fluorescence in situ hybridization (Q-FISH) was performed as described before (Gonzalo et al., 2006; Samper, Goytisolo, Slijepcevic, Van Buul, & Blasco, 2000) with some modifications: 1) Samples were not treated with pepsin and were subjected directly to dehydration steps. 2) Formamide concentration during incubation with the probe was reduced from 70% to 30%. 3) Samples were not subjected to 80ºC denaturation step. 4) Incubation time was reduced to 30 min. Samples were then washed with 4xSSC 0.05% Tween for 15 min (3x5min). Centromere (major satellite) PNA probes labeled with CY3 (Panagene) were used. Nuclei were counterstained in a 4 μg/ml DAPI/PBS solution before mounting with Vectashield (Vector Laboratories H-1000).

RESULTS

PART I

DEFINING A NOVEL (HA)PLOIDY CHECKPOINT

As we have exposed in the introduction, there is the general notion in the field that haploid mammalian cells spontaneously “diploidize”, explaining the progressive loss of haploid cells in culture. This suggests that haploidy might be an unstable genetic state although the reasons for this phenomenon remained largely unknown. Here, we present our findings regarding the underlying cause of “diploidization” and reveal two independent strategies capable of stabilizing haploidy in culture.

1. Haploid mammalian cells grow slower than diploids

Consistent with previous reports, while growing HAP1 cells we noticed a progressive loss of haploid cells (Figure 8A), eventually losing all haploid cells from the cultures if cell sorting was not performed on regular basis. To determine the basis of this phenomenon, we first explored whether haploid cells could have a growth disadvantage compared to diploid cells. To test this idea, haploid and diploid cell populations of HAP1 cells were sorted and cultured individually. In clonogenic assays, we observed that haploid cells formed smaller colonies compared to their diploid counterparts (Figure 8B, 8C). Moreover, in independent experiments the number of diploid cells after 6 days of culturing was almost five times higher when compared to the number of haploid cells (Figure 8D). These results indicated that haploid mammalian cells have a growth disadvantage compared to diploid cells existing in HAP1 cultures.

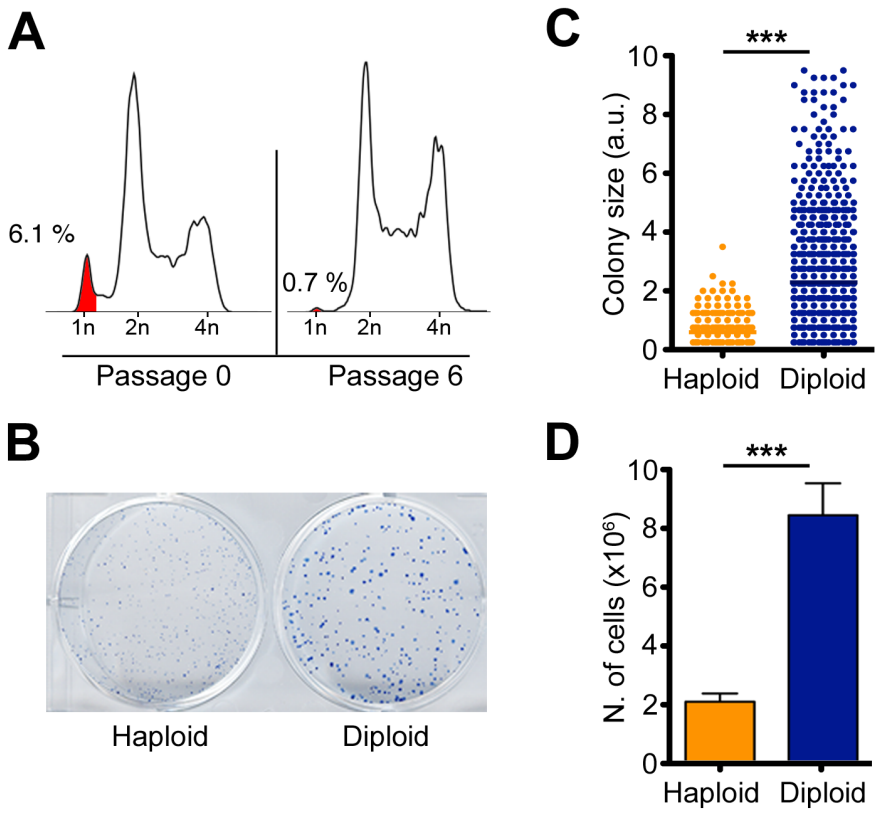


Figure 8: Diploid cells show a better growth fitness than haploids. (A) DNA content analyses by flow cytometry illustrate the rapid loss of the haploid population in early passages of HAP1 cells. Cells were stained with Hoechst. The percentage of haploid cells in G1 is represented in red. (B) Representative image of a methylene blue stained clonogenic assay of a haploid and diploid cell culture 5 days after plating. (C) Colony size of the clonogenic assay as shown in (B). Data represent two independent experiments with three technical replicates each. *** $P< 0.001$. (D) Total number of haploid and diploid cells 6 days after plating. As starting point, 5000 cells were seeded for each culture. Data represent two independent experiments with three technical replicates each. Error bars indicate SEM. *** $P< 0.001$.

We next asked whether the loss of haploid cells could be due to the better proliferation capacities of diploid cells, which would gradually take over the entire culture. To assess this possibility, sorted haploid HAP1 cells were infected with lentiviruses encoding for red fluorescent protein (RFP) and diploid cells with lentiviruses encoding for enhanced green fluorescent protein (EGFP). Subsequently, we mixed both populations at a 1:1 ratio and grew them for 36 days in culture. A time-course analysis by flow cytometry during the experiment revealed a gradual loss of the haploid population over time so that only 7% RFP positive cells remained at day 36, which were mostly haploid in DNA content (Figure 9A, 9B). Since the previous experiments indicated that haploid HAP1 cells were being out-competed by faster growing diploids, we speculated that cultures initiated from single-cell sorted haploid cells should remain haploid over time. Correspondingly, single-cell sorted HAP1 cell lines remained haploid even one month after the cell sorting (Figure 9C).

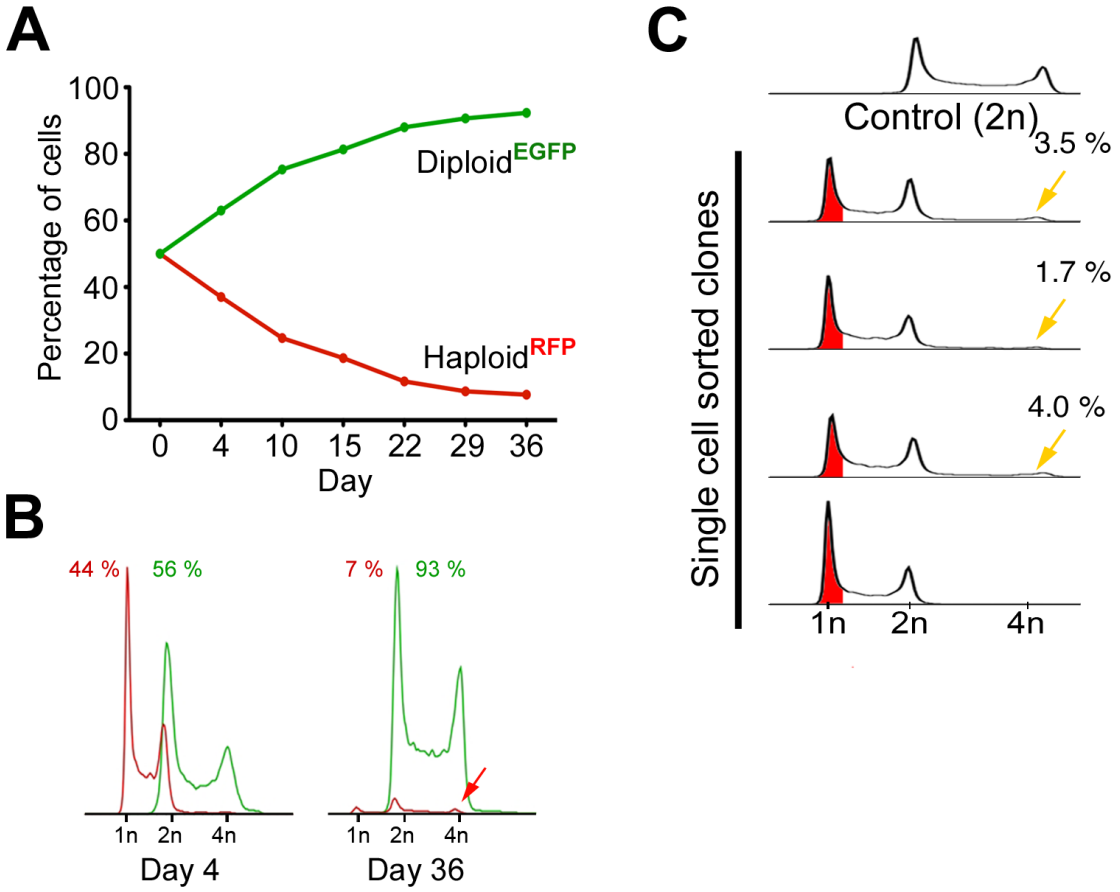


Figure 9: The loss of haploids in culture is due to the overgrowth of diploids. (A) Percentages of RFP-positive and EGFP-positive HAP1 cells measured by flow cytometry over a course of 36 days. The starting point of the culture was 50% for each population. (B) DNA content analysis from the two cell populations displayed in (A) at day 4 and 36. Red arrow points to the diploid 4n population found in RFP-positive HAP1 cells. (C) DNA content analysis by flow cytometry showing single-cell sorted clonal HAP1 cells at day 32 after cell sorting. In red is displayed the G1 peak of the haploid population. The yellow arrows point at the percentage of 4n population in each clone.

Of note, DNA content staining revealed that a small fraction of the single-cell sorted haploids had become diploid after one month in culture (Figure 9C), indicating that bona-fide diploidization also occurs in HAP1 cells. Nevertheless, despite the fact that diploidization does take place at a very low frequency, our results reveal that loss of haploidy in HAP1 cells is mostly due to an outgrowth by diploids existing in these cultures.

2. Loss of *P53* stabilizes haploidy in HAP1 cells

Besides haploidy, other alterations of ploidy in eukaryotes such as tetraploidy or aneuploidy have also been extensively documented to lead to reduced fitness (Ganem & Pellman, 2007; Santaguida & Amon, 2015), which in part is due to an activation of the tumor suppressor P53 (Ganem et al., 2014). Consequently, P53 deficiency is able to rescue the proliferation impairment of aneuploid and tetraploid mammalian cells (Cross et al., 1995; Fujiwara et al., 2005; M. Li et al., 2010; Thompson & Compton, 2010). Based on this, we first asked whether P53 levels were also altered in haploid cells. Indeed, P53 levels were higher in sorted haploid HAP1 cells compared to diploids (Figure 10A). Moreover, we detected a concomitant increase of the P53-target p21 in haploid cells indicative of an active P53-dependent transcriptional response. Consistently, P21 levels were abrogated by the expression of a *P53*-targeting shRNA (Figure 10A). Importantly, we noticed that 43 days after starting a culture, P53-depleted HAP1 cells contained a higher percentage of haploidy compared to control cells (Figure 10B), suggesting that P53 activation could play an important role in the poor growth of haploid HAP1 cells. To address this hypothesis, haploid-sorted HAP1 cells were transfected with plasmids expressing the Cas9 nuclease and a *P53*-targeting short guide RNAs (sgRNAs). 45 antibiotic-resistant clones were subsequently isolated, from which 21 still expressed P53 (wild type, WT) and 24 had lost P53 expression (knockout, KO) (Figure 10C). Remarkably, although many of the WT clones had undergone diploidization to various extents, the majority of the *P53*-KO clones (87.5%) remarkably remained haploid (Figure 10D). Next, we cultured haploid WT and *P53*-KO HAP1 clones for 2 months and monitored their ploidy status over time. Consistent with all previous data, P53-deficient clones maintained their haploid status significantly better compared to WT clones (Figure 10E, 10F).

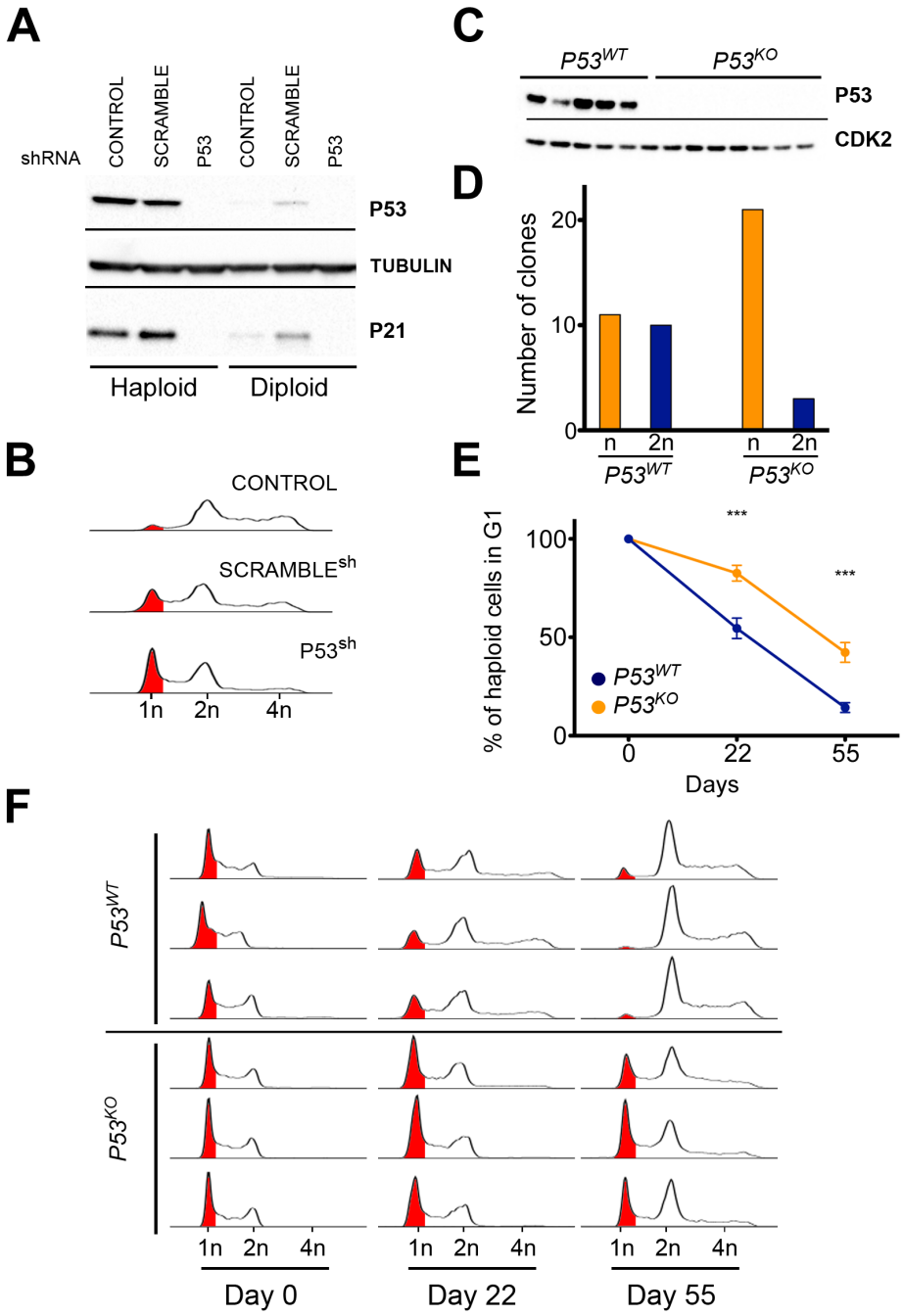


Figure 10: P53 depletion stabilizes haploidy. (A) P53 and P21 levels shown by Western blot in haploid and diploid cell populations infected with lentiviruses encoding for scramble or *P53*-specific shRNAs. Control cells were infected with empty vectors and expression of tubulin was used as a loading control. (B) DNA content analyses are displayed from the cell populations in (A) 43 days after infection and cell sorting. (C) A representative Western blot of the cell clones shown in (D) to illustrate P53-depletion by Cas9-gene editing in HAP1 clonal cell lines, CDK2 was used as loading control. (D) Graph displaying the number of isolated WT (n=21) and P53-deficient (n=24) clones obtained after the transfection of haploid HAP1 cells with a plasmid encoding for Cas9 and *P53*-targeting sgRNA. The DNA content was measured by flow cytometry and classified into haploid or diploid cell clones. (E) The percentage of haploid cells in G1 upon culture of the 11 WT and 21 P53-depleted HAP1 clones over 55 days is displayed in the graph. Error bars indicate SEM. *** $P < 0.001$. (F) DNA content analyses from 3 different WT and 3 different P53-depleted HAP1 clones from E are displayed at day 0, 22 and 55. The G1 haploid population is labeled in red.

Next, we assessed the relevance of two downstream targets of P53 in the maintenance of haploid phenotype; P21, a key mediator of P53-dependent cell cycle arrest (el-Deiry et al., 1993), and USP28, a deubiquitinase involved in the G1 arrest that follows a prolonged mitotic arrest (Meitinger et al., 2016). Both genes were deleted in HAP1 cells by CRISPR-mediated editing, and KO clones were followed in culture to evaluate their DNA content over time (Figure 11A, 11C). Interestingly, P21- or USP28-depleted cell pools lost the haploid cell fraction as fast as the WT clones (Figure 11B, 11D), which suggested that P53-dependent apoptosis, rather than cell cycle arrest, was likely responsible for the reduced fitness of haploid HAP1 cells.

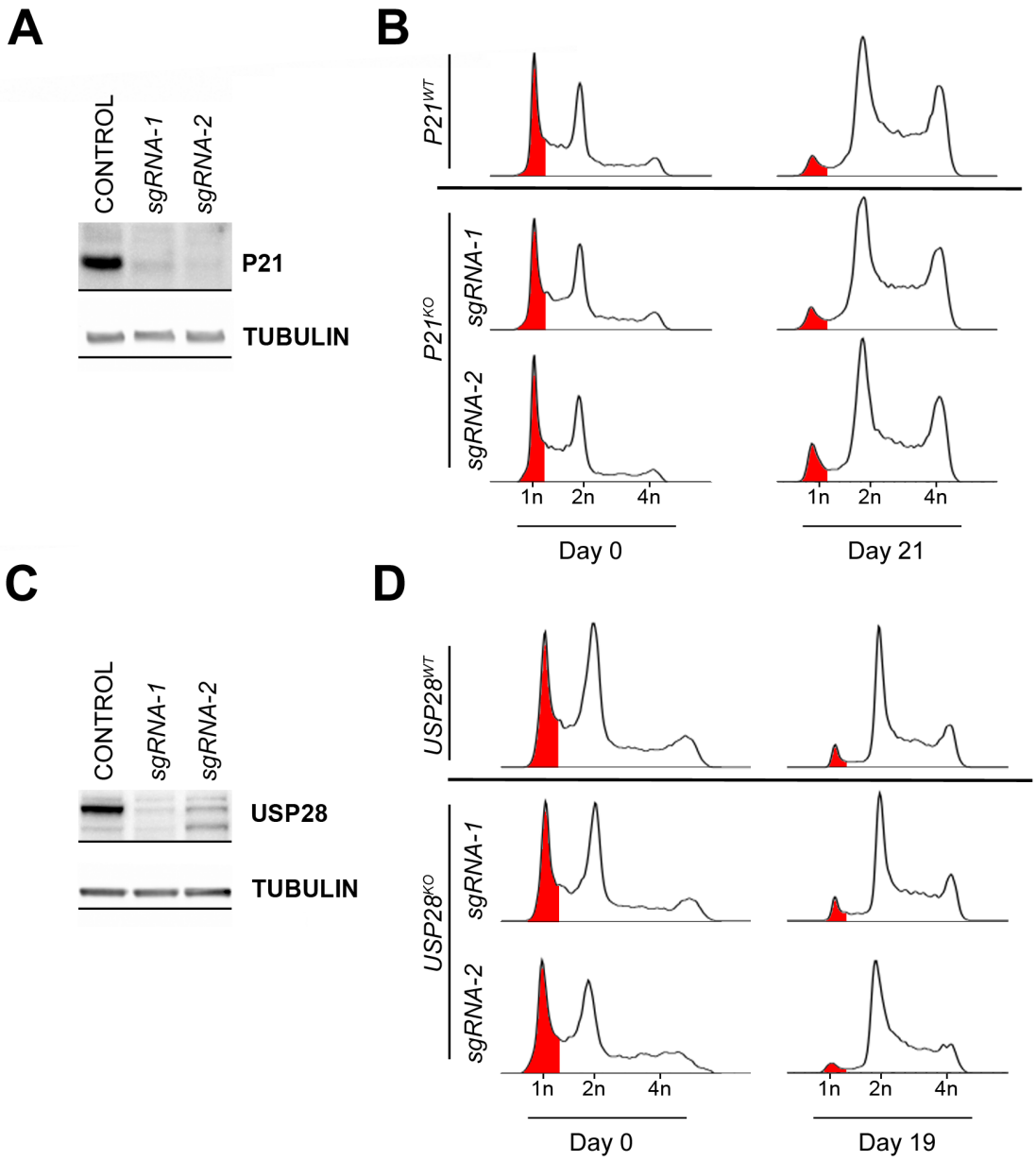


Figure 11: Haploidy is not stabilized by the depletion of P21 or USP28. (A, C) P21 (A) and USP28 (C) depletion in HAP1 cells after Cas9-dependent specific gene editing with 2 independent sgRNAs or empty control shown by Western blot. Tubulin serves as a loading control. (B) DNA content analyses from the samples described in (A) at day 0 and day 21; G1 haploid cells are labeled in red. (D) DNA content analyses from the samples described in (C) at day 0 and day 19; G1 haploid cells are labeled in red.

3. Absence of *P53* facilitates the generation and maintenance of mhaESCs

As described above, HAP1 cells derive from the human cancer cell line KBM7, which carries a high number of known and unknown mutations, which could in part contribute to P53 activation. Thus, in order to rule out that the effects we observed were not a HAP1-cell specific phenomenon, we next evaluated the role of P53 in primary mouse haploid embryonic stem cells (mhaESCs). First, we determined the levels of endogenous P53 by high throughput microcopy in a haploid and diploid cell populations sorted from a mixed population of mhaESCs. Similar to HAP1 cells, we observed a clear increase of P53 levels in mhaESCs compared to their diploid counterparts (Figure 12A).

Next, we checked if *P53* loss could facilitate the establishment and maintenance of newly generated mhaESC lines from activated oocytes. Thus, we derived WT and P53-deficient haploid ESC lines by inducing parthenogenesis with strontium chloride (U Elling et al., 2011; Martin Leeb & Wutz, 2012) on oocytes isolated from *P53*$^{-/+}$ female mice (Figure 12B, 12C). As mentioned in the introduction, the generation of mhaESCs is quite inefficient due to the low amount of haploid cells existing after ICM isolation and the very rapid loss of haploid cells in culture. The first DNA content analysis we could perform was at passage 5, at which we had enough cells for the analysis. While, as expected, we detected a very low amount of haploids in all WT cell lines, there was a clear increase in the percentage of haploids in all P53-deficient cell lines (Figure 12D). Further enrichment of the haploid cell population in P53-deficient cell lines could be obtained at later passages and after only 2 rounds of cell sorting. This was in contrast to the percentage of haploids observed in the WT cell lines, where only a minor enrichment of the initial haploid cell population was achieved after 2 rounds of cell sorting (Figure 12E). The results above suggested that, similar to what occurs in HAP1 cells, the loss of haploidy in mhaESC cultures could be due to a P53-dependent reduced fitness of haploid cells, which leads to the out-competition of the haploids by pre-existing diploids in these cultures. Consistently, single-cell sorting also facilitated the maintenance of WT mhaESCs (Figure 12F). Of note, spectral karyotype (SKY) analysis of these single-cell sorted clones confirmed a true haploid genome (Figure 12G).

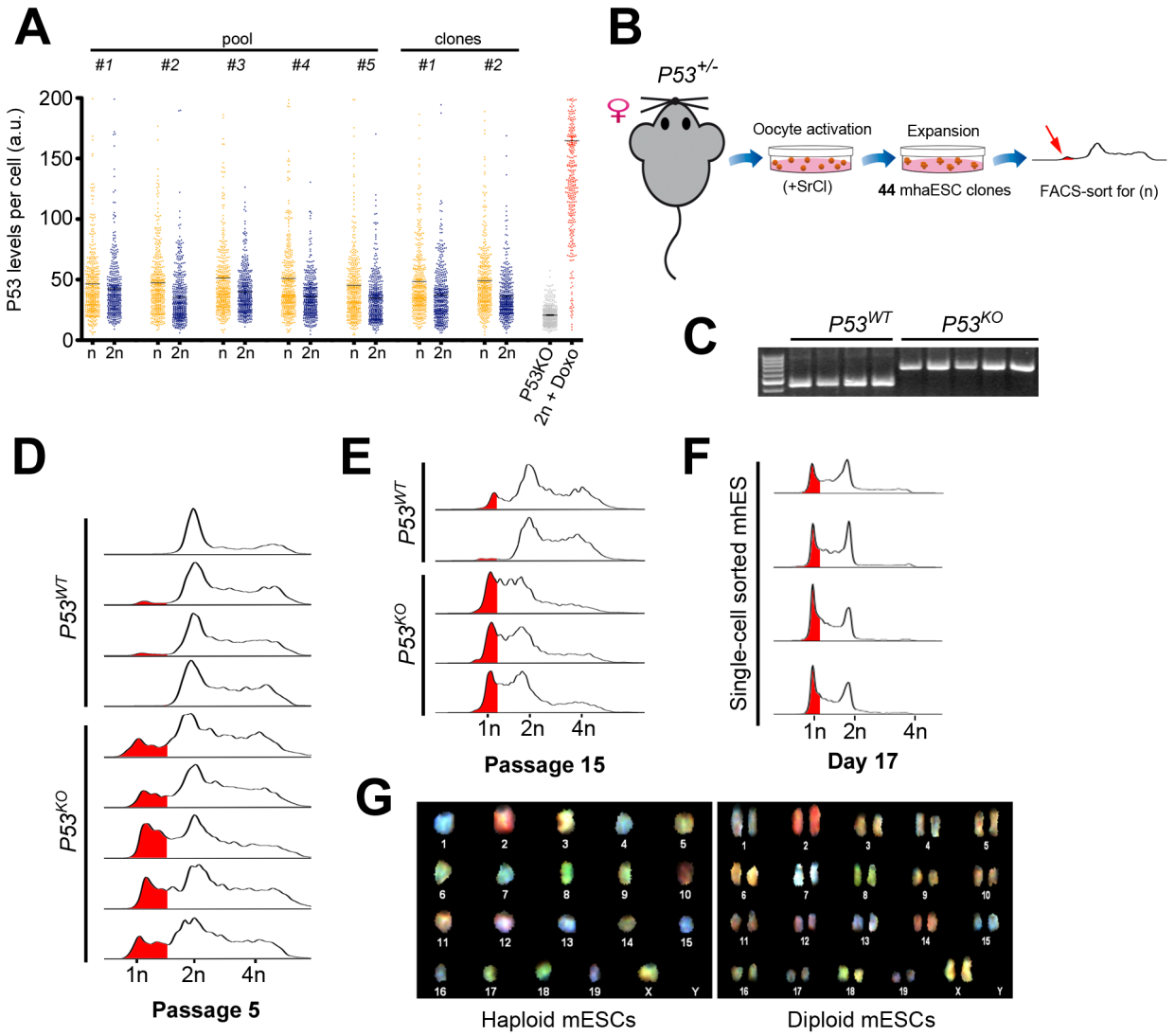


Figure 12: Depletion of *P53* facilitates the generation and maintenance of mhaESCs. (A) P53 levels were measured by high throughput microscopy in individual haploid and diploid cell lines that were sorted from pools or single-sorted WT mhaESC clones. P53-depleted ESCs and ESCs treated with Doxorubicin to induce P53 expression served as a staining control. (B) Schematic representation of the protocol to generate mhaESCs from *P53*$^{-/+}$ female mice. (C) Representative PCR analysis illustrating the *P53* status in newly generated mhaESC lines. (D) and (E) Representative DNA content analysis from WT and P53-deficient mhaESC lines at passage 5 (D) and passage 15 including two cell sorts to select for haploidy (E). G1 haploid cells are labeled in red. (F) DNA content analysis from WT single-cell sorted mhaESC clones at day 17 after sorting. G1 haploid cells are labeled in red. (G) Representative SKY analysis from a WT haploid and a diploid ESC clone.

Finally, we tested if P53 re-activation would be particularly toxic for haploid cells in P53-deficient mhaESC, or whether these cells had somehow adapted to the haploid state. In order to test this idea, we depleted P53 by expressing a Cre-dependent excisable *P53*-targeting shRNA cassette. Although, as expected, P53 depletion led to a better maintenance of haploidy in ESCs, Cre-dependent excision of the shRNA cassette restored P53 levels and led to a reduction of the haploid cells (Figure 13A, 13B). Thus, P53-deficient haploid ESCs do not “adapt” to the haploid

state, and remain sensitive to P53 activation. Taken all together, our data reveal that the rapid reduction of haploidy in mhaESC cultures is due to an overgrowth of diploid ESCs. In addition, and similar to what we observed in HAP1 cells, P53 depletion or single-cell sorting stabilizes the haploid state in mhaESC lines.

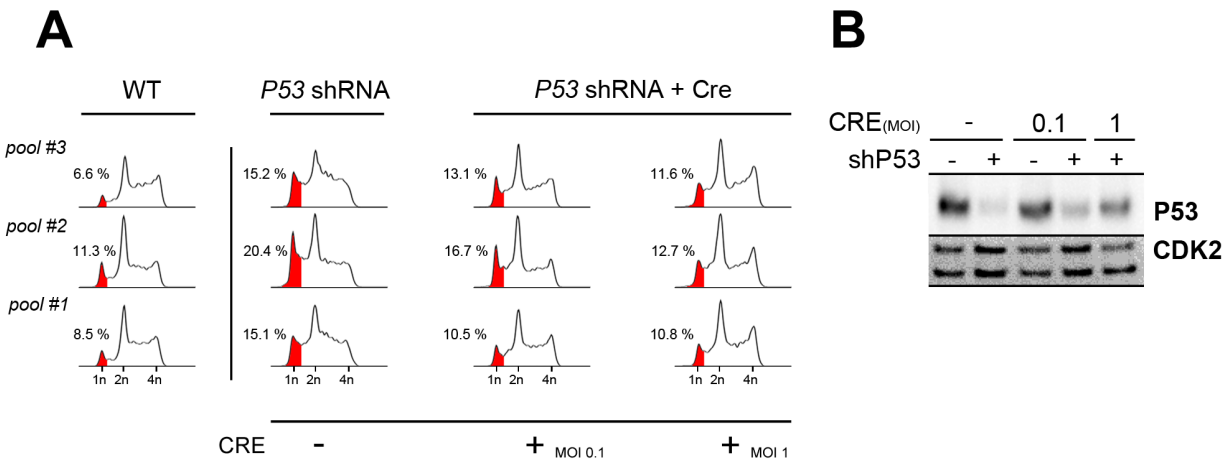


Figure 13: P53-depletion does not induce an adaptive response to maintain haploidy in ESCs. (A) DNA content analysis from 3 independent mhaESC cultures infected with a Cre-dependent excisable *P53*-targeting shRNA cassette and the subsequent restoration of P53 levels after infection with Adeno-Cre 2 days later. Uninfected cell cultures served as a control. G1 haploid cells are labeled in red and the numbers indicate its percentage. (B) Western blot showing the P53 levels in the different conditions from (A).

4. P53-dependent cell death limits the expansion of mhaESCs in culture

We next explored the underlying cause of the poor growth of WT mhaESCs. We reasoned that because ESCs lack a P53-dependent G1/S checkpoint (Aladjem et al., 1998; Hong & Stambrook, 2004), a reduced S-phase entry might not explain the impaired growth of mhaESCs. Accordingly, when we compared the percentage of replicating cells by evaluating the incorporation of ethynyl dexoyuridine (EdU) we observed a similar percentage of EdU incorporation rates in haploid and diploid cultures (Figure 14).

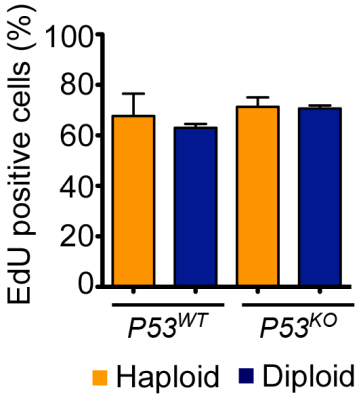


Figure 14: Haploid and diploid ESCs display a similar number of replicating cells in culture. Percentage of EdU-positive WT and P53-deficient haploid and diploid mESCs after a 30 min EdU pulse. Three independent single-cell sorted haploid and diploid cell clones were evaluated in duplicates and averaged. Error bars indicate SEM.

To further determine the reasons behind the loss of haploid mhaESCs in culture, we infected P53 WT and KO single-cell sorted mhaESCs with lentiviruses expressing a fusion protein between EGFP and histone H2B (H2B-GFP) to record videos and monitor cell behavior for 24h (Figure 15A, 15B). This experiment revealed several interesting findings: First, we detected a similar amount of cells in mitosis, consistent with the previous observation that cell cycle progression was not grossly affected in haploid or diploid mhaESCs. Second, we observed a clear increase in cell death in the haploid WT cultures with up to 56.36% cells dying in 24h (compared to 7% in the corresponding diploid WT cultures). Third, P53-deficiency significantly rescued the viability of haploid mhaESCs. Fourth, we noticed that WT haploid cells mostly died within or shortly after mitosis, suggesting that the cell death could be associated to problems during chromosome segregation. This observation is also supported by the fact that mitosis was longer in haploid cells compared to diploid cells (Figure 15B). Moreover, image analysis revealed that of up to 18 % of the haploid mitosis presented lagging chromosomes, anaphase bridges or micronuclei. While P53-deficient haploid cells displayed a similar amount of segregation problems in mitosis, the absence of P53 limited their cell death. Taken together, these data indicated that haploid mhaESCs are lost in culture due to chromosome segregation problems that lead to the activation of P53-dependent cell death.

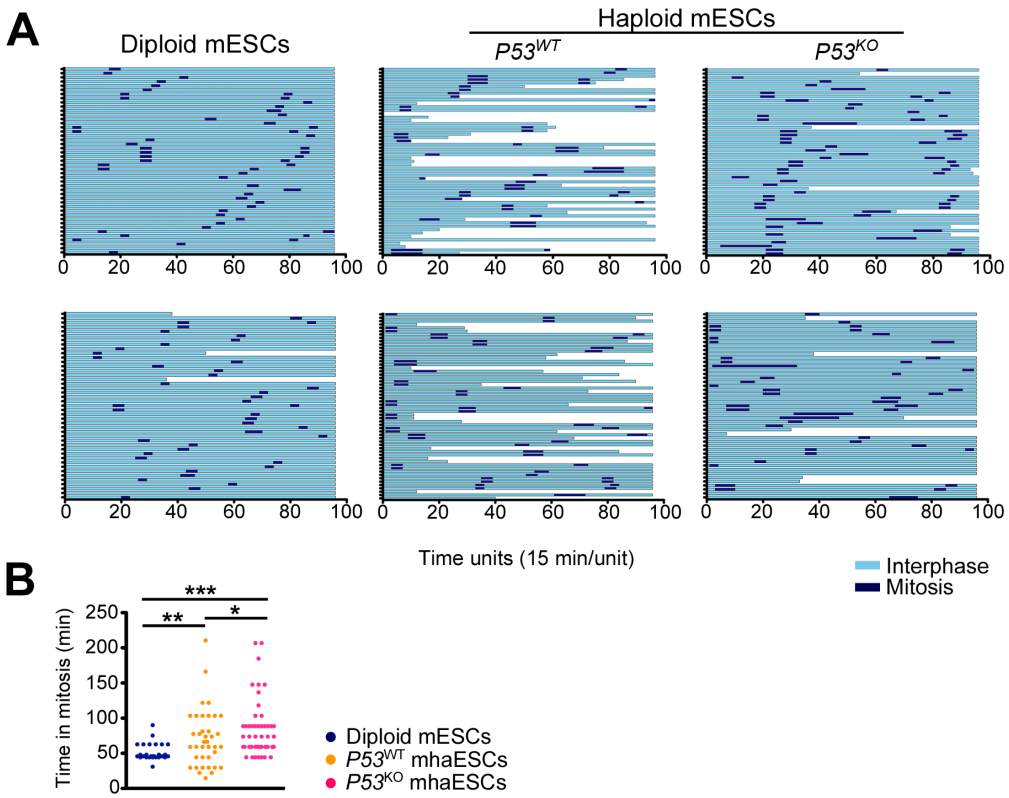


Figure 15: P53-dependent death limits the expansion of mhaESCs. (A) Schematic representation of time spent in mitosis by individual, EGFP-H2B expressing, single-cell sorted WT haploid, P53-deficient haploid and WT diploid ESCs. Each scheme represents a single-cell sorted clonal cell line and horizontal bars depict the life of an individual cell showing in light blue the time in interphase and in dark blue the time in mitosis. Time in mitosis was defined as the time between chromosome condensation and cytokinesis. Cells were monitored every 15 min for a total of 24 h and a minimum of 40 cells per condition was analyzed. (B) Time spent in mitosis from the single-cell sorted clones shown in B. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

5. Chromosome segregation defects in mhaESCs

Consistent with the hypothesis that the P53-dependent cell death observed in haploid ESCs was due to chromosome segregation problems, we observed a differential sensitivity to the microtubule poison Taxol in WT mhaESCs, which was abolished in the absence of P53 (Figure 16A). To investigate the reasons that could underlie the segregation problems of mhaESCs, we first explored if these could arise from increased replication stress (RS), a type of DNA damage occurring during S-phase when fork progression is altered. Previous works had revealed that, upon the presence of RS, cells could enter into mitosis without a fully replicated genome and therefore lead to severe chromosome segregation problems (Mankouri, Huttner, & Hickson, 2013). To test whether mhaESCs suffered from higher levels of RS and consequently from higher levels of DNA damage, we measured the number of 53BP1 foci and the intensity of pan-nuclear histone H2AX phosphorylation in the nucleus (Figure 16B, 16C). While we could not detect a difference in any of the parameters, with these approaches we cannot exclude the possibility that specific regions of the haploid genome might suffer by RS and remain unreplicated before they reach mitosis.

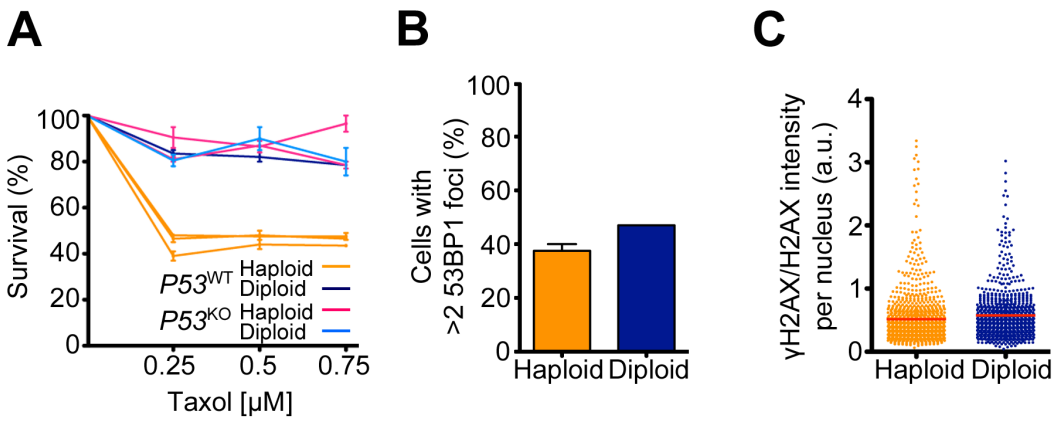


Figure 16: WT mhaESCs are sensitive to the spindle poison Taxol and do not suffer from RS. (A) WT and P53-deficient single-cell sorted haploid and diploid ESCs were treated with various doses of Taxol for 24h, when cell viability was measured. (B) 53BP1 foci were counted in haploid and diploid ESCs, percentage of cells with more than two foci are shown. (C) High throughput analysis of γH2AX intensity per nucleus normalized by the total levels of H2AX in WT haploid and diploid ESCs. Centerlines indicate mean values.

We next explored if the segregation defects observed in WT mhaESCs arise from problems directly occurring in mitosis. In this regard, we first tested if mhaESCs lacked centrosomes, since these cells derive from parthenogenic oocytes, and in nature centrosomes are provided by the sperm during fertilization (Simerly et al., 1995). Interestingly, immunofluorescence analysis of γTubulin expression, a centrosomal marker, revealed normal numbers of centrosomes in mhaESCs (Figure 17A, 17B), revealing that centrosomes are formed *de novo* in mhaESCs.

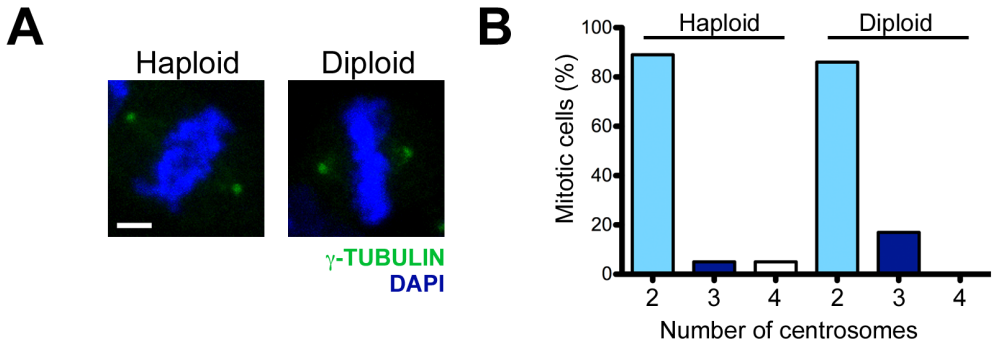


Figure 17: Normal centrosome numbers in mhaESCs. (A) Representative immunofluorescence images of WT haploid and diploid ESCs in metaphase visualizing the centrosomes with γTubulin in green, DNA is stained with DAPI in blue. (B) Quantification of mitotic cells with 2, 3 or 4 centrosomes counted in WT haploid and diploid ESCs.

Next, we tested whether imbalanced ratios between the spindle size and DNA content could underlie this phenomenon. In fact, an overlay of 25 spindles showed that while all spindles from diploid ESCs were perfectly aligned, the overlay from haploid spindles led to a blurry picture indicating a noisy distribution (Figure 18A). Moreover, although haploid cells contain half of the DNA than diploids (Figure 18B-D), the distribution of the DNA at the metaphase plate was wider and more heterogeneous in mhaESCs compared to the diploids (Figure 18E, 18F), further underscoring the intrinsic difficulties of haploid cells to arrange their chromosomes during mitosis (Figure 18A).

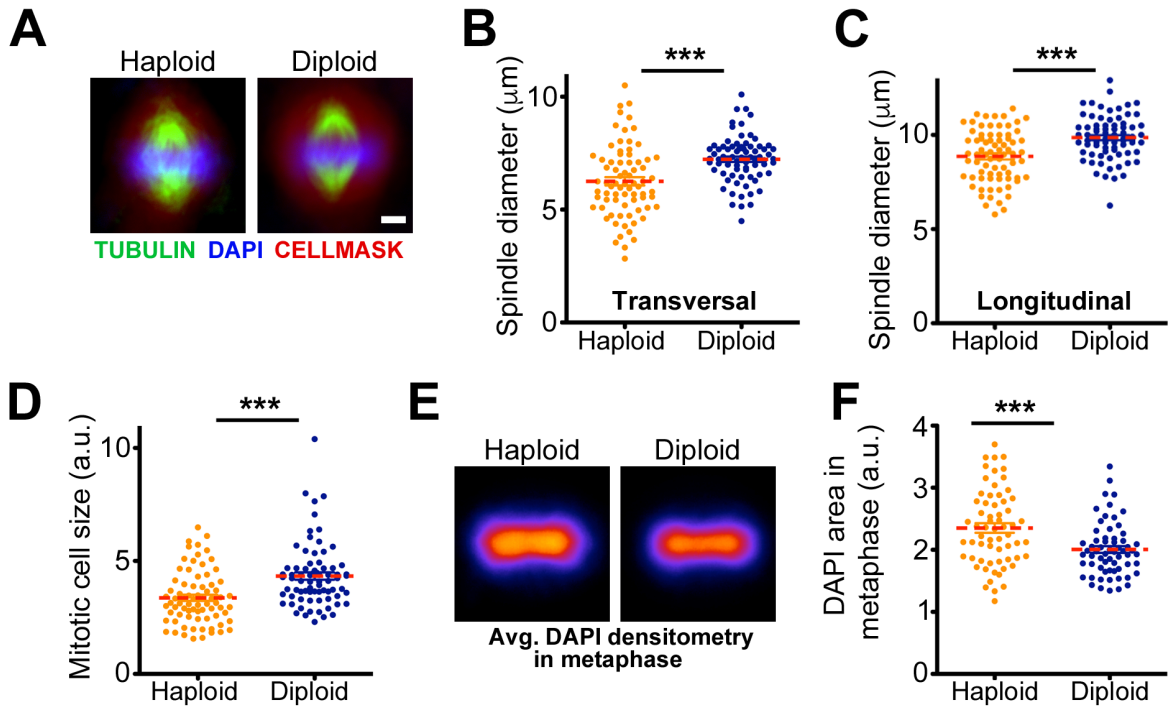


Figure 18: Heterogeneity in the alignment of the mitotic spindle in mhaESCs. (A) Representative images show an overlay of 25 different metaphases from WT haploid or diploid ESCs. Tubulin was stained in green to visualize the spindle, DNA in blue was stained with DAPI and the cytoplasm was stained in red with Cellmask. One single-cell sorted ESC clone each is shown but three different ones were analyzed per condition. (B-D) Transversal (B) and longitudinal (C) spindle diameter lengths, as well as mitotic cell size (D) was measured in 25 metaphases from WT haploid or diploid ESCs. Data from one single-cell sorted ESC clone each is shown but three different ones were analyzed in total per condition. (E) Representative images show the overlay of the average DAPI densitometry, hence the distribution of the DNA, from 25 different metaphases from WT haploid or diploid ESCs. One single-cell sorted ESC clone each is shown but three different ones were analyzed per condition. (F) DAPI area was measured in 25 metaphases from WT haploid or diploid mouse ESCs. Data from one single-cell sorted ESC clone each is shown but three different ones were in total analyzed per condition.

6. Generation of haploid mouse tissue

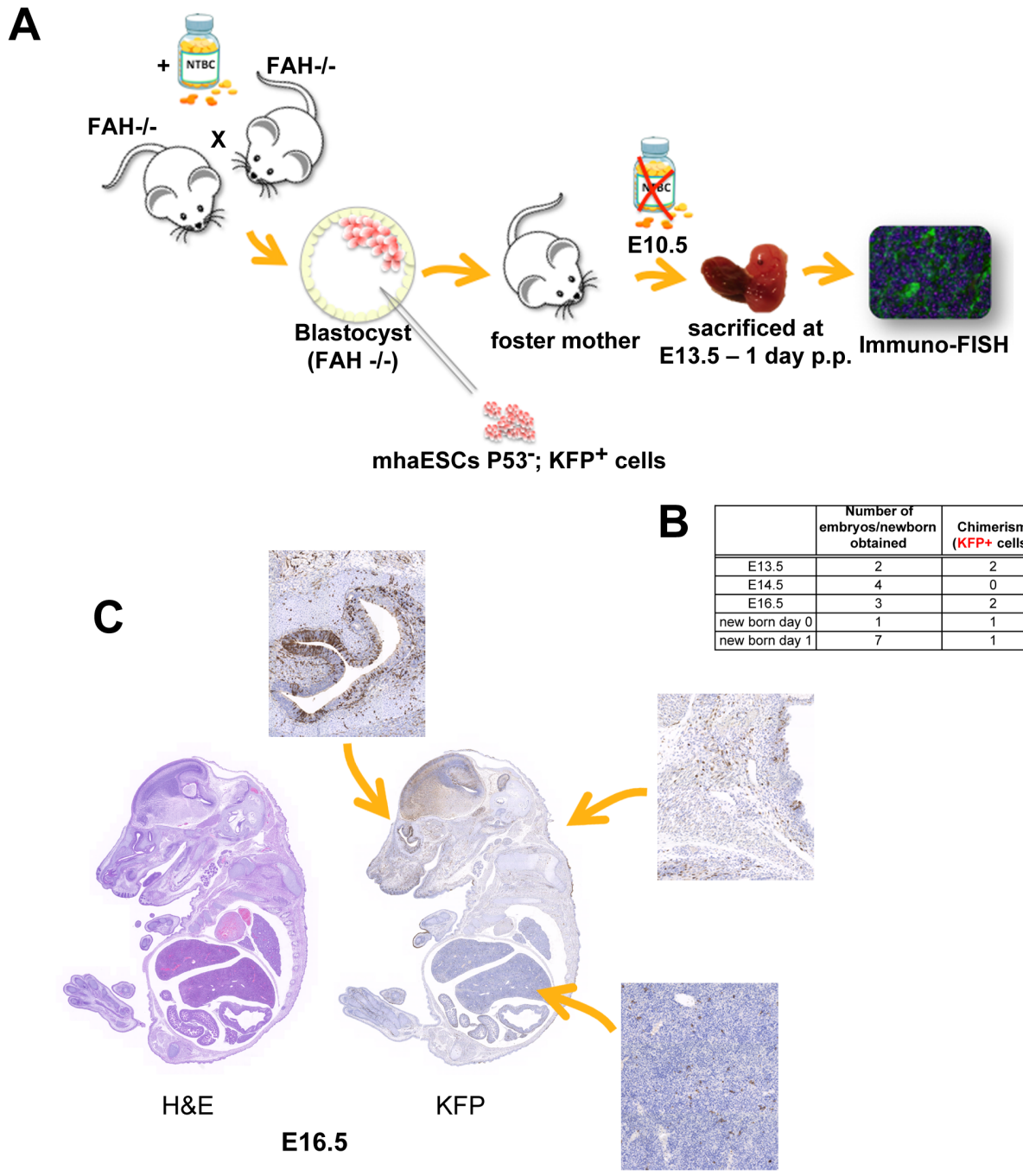
A long-standing question regarding mhaESCs refers to whether they can contribute to the generation of haploid tissue. The current literature shows that although mhaESCs can contribute to the generation of mouse chimeras, the tissue obtained contained a diploid genome (U Elling et al., 2011; M. Leeb et al., 2012; Martin Leeb & Wutz, 2012), reinforcing the idea that haploidy is also a very unstable genetic state *in vivo*. Nevertheless, based on our results presented above, we speculated that the use of P53-deficient mouse mhaESCs could help to stabilize haploidy during differentiation and perhaps allow the formation of haploid tissue. In collaboration with the Transgenic Mouse Unit at the CNIO, we conducted a wide variety of approaches (summarized in Table 1) to explore this possibility.

#1	Microinjections of a pool of P53-deficient mhaESCs into $EGFP^{+}$ blastocysts
#2	Microinjections of single-cell sorted P53-deficient mhaESCs into $EGFP^{+}$ blastocysts
#3	Microinjections of single-cell sorted P53-deficient mhaESCs into KFP^{T} blastocysts

Table 1: Different strategies performed trying to obtain haploid mouse tissue.

In order to easily identify the haploid derivatives, we microinjected P53-deficient mhaESCs into blastocysts obtained from fluorescently labeled mice. Unfortunately, we could not detect clear chimerism in the embryos obtained from any of these initial experiments. Next, we changed our initial experimental setup by fluorescently labeling the mhaESCs, as it could facilitate the detection of the haploid derived cells. To do so, $P53^{-/-}$ male mice were crossed with females expressing the fluorescent reporter Katushka ($KFP^{+/T}$) (Diéguez-Hurtado et al., 2011). Subsequently, $P53^{+/-}/KFP^{+/T}$ females were used as oocyte donors for the generation of parthenogenetic mhaESCs. To further increase our chances of developing haploid mammalian tissue, we speculated that focusing on organs that physiologically contain polyploidy cells, such as the liver (Gentric & Desdouets, 2014), could increase the probability of tolerating haploid differentiated cells. Hence, we took advantage of mice lacking the tyrosine catabolic enzyme fumarylacetoacetate hydrolase (FAH) and immunodeficient for T and B cells ($Rag2^{-/-}$; $Il2rg^{-/-}$), which are frequently used in transplantation studies (Azuma et al., 2007; Espejel et al., 2010). Mice lacking FAH develop severe hepatocyte damage and die from liver failure within the first weeks after birth, unless they are constantly supplemented with 2-(2-nitro-4-trifluoromethylbenzoyl)-1-3-cyclohexanodione (NTBC), a potent inhibitor of 4-hydroxyphenylpyruvate dioxygenase (Azuma et al., 2007; Espejel et al., 2010). Microinjecting wild type induced pluripotent stem (iPSCs) cells in FAH-depleted blastocysts leads to fully viable chimeric mice, where most of the liver is derived from fully functional hepatocytes differentiated from iPSCs cells (Espejel et al., 2010). Thus, we hypothesized that a similar

approach but performed with *P53*$^{-}$; *KFP*^{T} mhaESCs could lead to viable mice with livers made to a large extent from mhaESCs-derived cells (Figure 19A).



	Number of embryos/newborn obtained	Chimerism (KFP+ cells)
E13.5	2	2
E14.5	4	0
E16.5	3	2
new born day 0	1	1
new born day 1	7	1

Figure 19: Generation of *P53*$^{-}$; *KFP*$^{+}$ mhaESCs chimeras. (A) Schematic representation of the experimental procedure to obtain chimeras from P53-deficient; KFP^{+} mhaESCs. (B) Summary of the embryos/newborns obtained from several microinjection sessions. (C) Histopathology slides of the chimeric embryo obtain at E16.5 stained with H&E (left) and anti-KFP (right, brown cells), representative areas are magnified (face, backskin, liver). Most of the chimerism was found in the head area.

To reduce the initial selection pressure, we maintained the foster FAH-deficient mother on NTBC until E10.5, where the liver starts to develop (Swartley, Foley, Livingston, Cullen, & Elmore, 2016). In collaboration with the Transgenic Mouse Unit we performed several microinjections with sorted *P53*$^{-}$; *KFP*^{T} mhaESCs into FAH-depleted blastocysts and obtained embryos at different developmental stages (Figure 19B). To evaluate the contribution of the *P53*$^{-}$; *KFP*^{T} mhaESCs we performed whole embryo immunohistochemistry analysis to detect KFP expression. While few embryos showed evidences of significant chimerism, at E16.5 presented an important contribution from the haploid cells in most tissues, including the liver (Figure 19C).

Next, to determine whether cells arising from *P53*$^{-}$; *KFP*$^{+}$ mhaESCs remained haploid after embryonic development or had diploidized, we performed, in collaboration with Paula Martínez (Telomeres and Telomerase Group, CNIO), immunofluorescence against KFP followed by quantitative fluorescence in situ hybridization (Q-FISH) to detect centromeric major satellite repeats (Figure 20A). We then quantified the averaged intensity of the FISH signal from centromeric repeats in KFP positive and negative cells within the same tissue as an indirect readout of cell ploidy. Interestingly, these analyses revealed that in certain tissues such as the dermis, KFP positive cells showed significantly lower levels of centromere intensity than KFP negative cells (Figure 20B, 20C). While these experiments might indicate that we have succeeded in generating haploid mammalian tissue, a formal proof for the existence of haploid cells in mouse embryonic tissues demands additional experiments, which are currently underway.

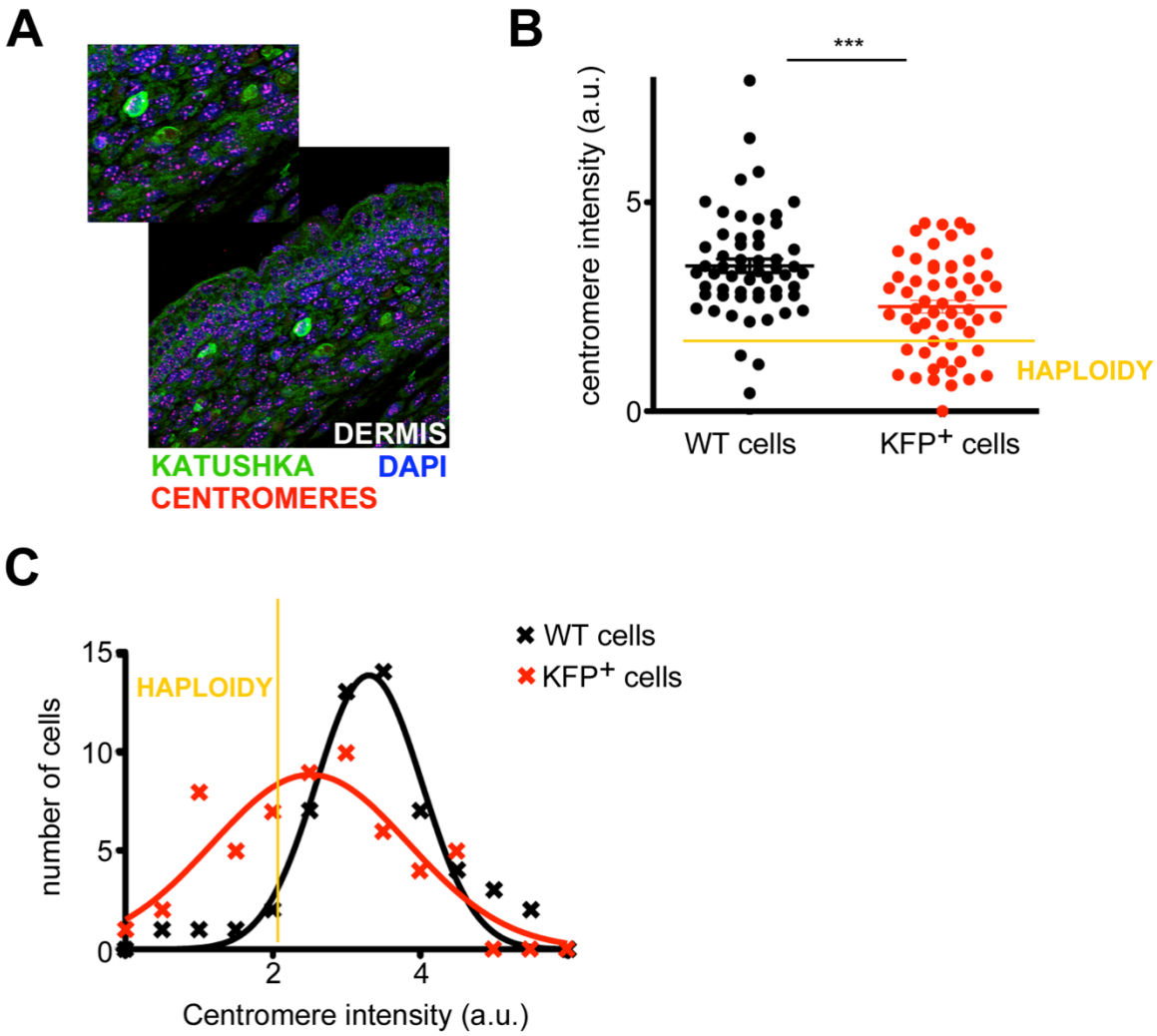


Figure 20: Evidence for haploid differentiated cells *in vivo*. (A) Representative picture of the Immuno-Q-FISH in the dermis of the embryo from Figure 19C. Katushka (KFP) in green allows the detection of P53-deficient KFP$^+$ differentiated cells. The centromeres were labeled in red and the nuclei with Dapi in blue. (B) Quantification of the centromere intensity in KFP$^+$ and WT cells in the dermis area. Haploid cells are expected below the yellow bar. 57 cells are displayed per column. ****P*< 0.001. (C) Histogram of KFP$^+$ and WT cells in the dermis showing the cell distribution dependent on the centromere intensity. Haploid cells are expected on the left side from the yellow bar. 57 cells are displayed per curve.

PART II

CHEMICAL STABILIZATION OF HAPLOIDY IN MAMMALIAN CELLS

Following our previous genetic work on the mechanisms that mediate the loss of haploid cells in culture, we next sought to conduct a chemical screening to identify compounds capable of stabilizing and/or favoring haploidy. Our original aim was to discover compounds that, when supplemented into the growth media, could facilitate the maintenance of haploid cells. In addition, this chemical biology approach could also help to understand better the underlying biology of haploid mammalian cells. In the following section, I present the results and what we learned from such screen.

1. A chemical screen searching for compounds favoring haploid or diploid cells

To identify chemical compounds that selectively favor either the haploid or the diploid genomic cell state, we first fluorescently labeled haploid HAP1 cells by expressing the red fluorescent protein tdTomato (TOM) and diploid HAP1 cells by expressing the enhanced green fluorescent protein (EGFP). Haploid^{TOM} and diploid^{EGFP} cells were then mixed in a 4:1 ratio, plated in duplicates in 96-well plates and treated individually for three weeks with a total number of 987 compound (FDA-approved Drug Library, Selleckchem, Table 2). Due to the long duration of this experiment, cells were splitted once and fresh compounds were added twice per week. At the endpoint, cells were trypsinized and analyzed by high throughput flow cytometry to determine the final TOM/EGFP ratio. Consistent with our previous work, the amount of haploid cells in control (DMSO-treated) wells was reduced to more than half after 3 weeks, with the consequent increase in the diploid cell population (Figure 21A). Taking this into account, we considered a compound as a "haploid hit" when the deviation to the percentage of haploid cells in control wells was higher than 30 % and a diploid hit in the opposite situation (Figure 21A, 21B). In the primary screen we identified a total number of 19 compounds favoring haploidy and 18 favoring diploidy. However, since the readout used was based on tdTomato/EGFP ratios and not DNA content, we next performed a detailed evaluation of the initial hits using the DNA content dye Hoechst as a direct readout for ploidy. For this, we first used freshly cell-sorted haploid HAP1 cells and treated them with the 19 haploid and 18 diploid hits for up to four weeks. From two independent validation rounds, we could finally verify 6 compounds that consistently increased the fraction of haploid cells (Figure 21C) and 5 compounds favoring the diploid state (Figure 21D).

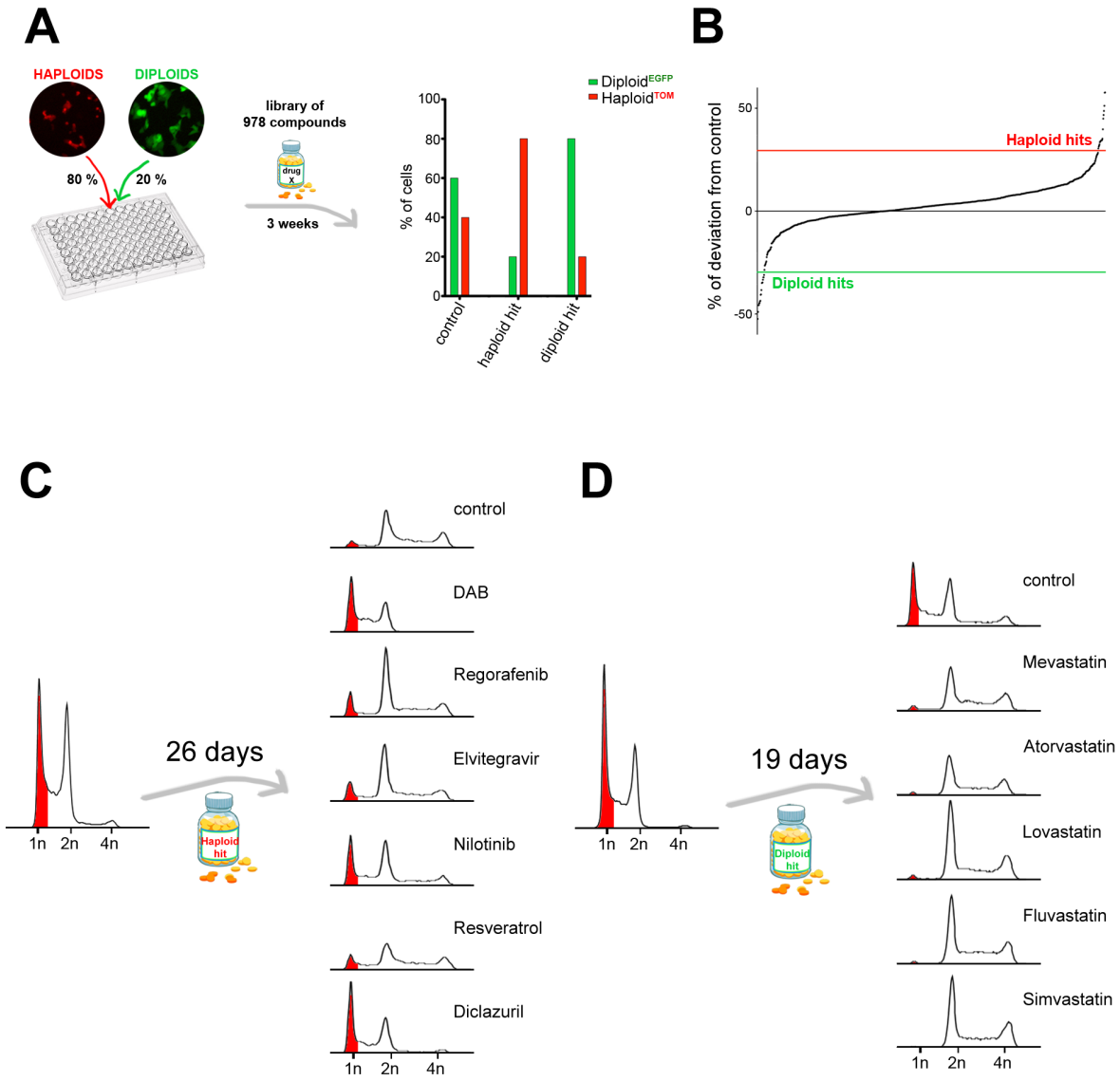


Figure 21: Chemical screening to identify compounds stabilizing haploidy or diploidy. (A) Schematic representation of the screening procedure. tdTomato-expressing haploid and EGFP-expressing diploid HAP1 cells were mixed in a ratio of 4:1 and further cultured in media supplemented with 978 FDA-approved drugs during the course of 3 weeks. Screening evaluation was performed by high throughput flow cytometry. (B) Summary graph representing the percentage of deviation compared to DMSO-treated control cells for the 978 compounds evaluated. Every dot represents one compound and 0% deviation represents no change compared to the control. A compound was classified as haploid hit when a total deviation of 30% or higher of tdTomato-positive cells compared to the control was found at the end of the screening. A compound was classified as diploid hit when a total deviation of 30% or lower of tdTomato-positive cells compared to the control was found at the end of the screening. (C) Validation of haploid hits by DNA content staining in haploid HAP1 cells shown before and 26 days after drug treatment. G1 haploid cells are labeled in red. (D) Validation of diploid hits by DNA content staining in haploid HAP1 cells shown before and 19 days after drug treatment. G1 haploid cells are labeled in red.

Among the compounds stabilizing haploidy we found, in a ranked order: 1) DAB (10-Deacetylbaccatin-III), a precursor of the chemotherapeutic drug Paclitaxel with unknown target (Guéritte-Voegelein, Sénilh, David, Guénard, & Potier, 1986; B. J. Li et al., 2017); 2) Regorafenib, a multikinase inhibitor used in several cancer treatments (Abou-Elkacem et al., 2013; Bruix et al., 2017; Demetri et al., 2013; Grothey et al., 2013; Wilhelm et al., 2011); 3) Elvitegravir, an integrase inhibitor of the human immunodeficiency virus (HIV) (Shimura et al., 2008); 4) Nilotinib, a clinically approved inhibitor of the tyrosine kinase cAbl for the treatment of chronic myeloid leukemia (Wyse, Brundin, & Sherer, 2016); 5) Resveratrol, a compound originally thought to activate SIRT1, that has been explored to treat diseases affected by abnormal metabolic control, inflammation and cell cycle defects (Berman, Motechin, Wiesenfeld, & Holz, 2017); and 6) Diclazuril, an anticoccidial used in the prevention of bovine coccidiosis (Zechner et al., 2015). Remarkably, all 5 compounds favoring the diploid state (Mevastatin, Lovastatin, Fluvastatin, Atorvastatin and Simvastatin) target the same enzyme (Hydroxymethyl glutaryl coenzyme A (HMG-CoA) reductase) and belong to the class of statins. These compounds are in the clinic as one of the most commonly prescribed medications worldwide to reduce blood cholesterol levels (Ramkumar, Raghunath, & Raghunath, 2016).

2. DAB selects for cells with lower ploidy

Given that DAB was the compound showing the highest stabilization of haploidy in HAP1 cells, we next investigated the resons behind this phenomenon. First, in order to further evaluate the potency of DAB as a stabilizer of haploidy, we mixed haploid^{TOM} and diploid^{EGFP} HAP1 cells in a 1:4 ratio. This initial distribution led to a complete loss of the haploid^{TOM} cell population in only few days. Remarkably, the DAB treatment not only maintained the haploid^{TOM} cell population, but this actually increased to up to almost 50% (Figure 22A). This result suggested that diploid cells are preferentially affected by DAB and are lost during the course of our experiment. Given the previous experiments, we next investigated if the effects of DAB on mixed haploid/diploid cultures could reflect a more general role of this drug in selecting for cells with a lower ploidy. As mentioned in the introduction, the HAP1 line derives from KBM7 cells, which in the patient originally contained a mix of near haploid and hyperdiploid cell clones (Andersson et al., 1995; Carette et al., 2011; Kotecki et al., 1999). Similarly, cell sorting of early passage HAP1 cells allowed us to isolate, on top of the haploid and diploid cell populations, a tetraploid HAP1 cell population. To test our hypothesis, we first cultured haploid and tetraploid HAP1 cell populations with DAB for 20 days. Flow cytometry analysis revealed that while haploidy is clearly maintained upon DAB treatment, near-tetraploid cells were particularly sensitive to his compound. In fact, the few surviving cells from the tetraploid culture contained a diploid genome, likely representing cell contaminants from the cell sorter that

were enriched during the experiment (Figure 22B). To further test a potential ploidy-dependent toxicity of DAB, we infected tetraploid HAP1 cells with lentiviruses encoding for blue fluorescent protein (BFP) and mixed them with haploid^{TOM} and diploid^{EGFP} HAP1 at a ratio of 1:1:1. Consistent with the lower fitness of haploid and tetraploid cells (Andreassen, Lohez, Lacroix, & Margolis, 2001; Ganem & Pellman, 2007; Olbrich et al., 2017), haploid^{TOM} and tetraploid^{BFP} HAP1 cells gradually disappeared from the mixed cultures while diploid^{EGFP} HAP1 cells became enriched. In contrast, DAB treatment strongly favored the haploid^{TOM} HAP1 cell population while decreasing the growth of diploid^{EGFP} cells and more remarkably, led to the virtual disappearance of the tetraploid^{BFP} HAP1 cell population (Figure 22C).

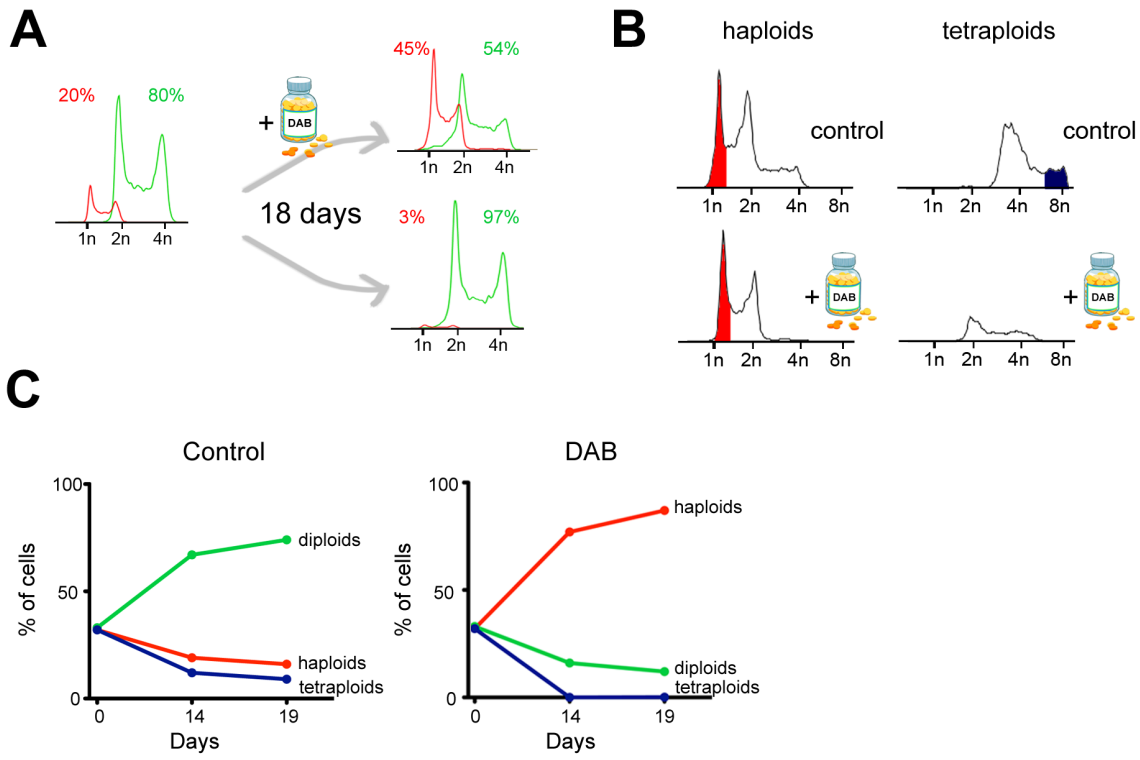


Figure 22: DAB selects for cells with lower ploidy. (A) tdTomato-expressing haploid and EGFP-expressing diploid HAP1 cells were mixed in a ratio 1:4 and cultured untreated or treated with 10 µM DAB for 18 days. DNA content analyses were performed at day 0 and day 18. (B) DNA content analysis of haploid, diploid and tetraploid HAP1 cells untreated or treated with 20 µM DAB treatment for 20 days. G1 haploid cells are labeled in red, G2 tetraploid cells are labeled in dark blue. (C) tdTomato-expressing haploid, EGFP-expressing diploid and BFP-expressing tetraploid HAP1 cells were mixed in a ratio of 1:1:1 and cultured untreated or treated with 10 µM DAB for 19 days. Percentage of cell population was evaluated by flow cytometry.

We next explored if the effects of DAB in selecting for lower ploidy were conserved in other cell types. For this, sorted mhaESCs cultures were treated with DMSO or DAB for a total of 14 days. Similar to HAP1 cells, a continuous DAB treatment in mhaESCs helped to maintain the haploid population (Figure 23A). Next, we took advantage of the spontaneous appearance of tetraploid mouse embryonic fibroblasts (MEFs) in SV40-transformed diploid cultures (Ganem &

Pellman, 2007). Diploid MEFs expressing EGFP and tetraploid MEFs expressing tdTomato were mixed at a 1:4 ratio. In agreement with our previous results, DAB treatment of the mixed culture for a total of 13 days showed a dramatic decrease of the tetraploid^{TOM} cell population (Figure 23B). Finally, we also tested DAB toxicity on diploid and tetraploid iPSCs. Similar to MEFs, we infected the diploid iPSCs with lentiviruses encoding for EGFP and the tetraploid iPSCs with lentiviruses encoding for tdTomato and mixed them in a 1:3 ratio. Once again, DAB treatment of the mixed culture for 6 days showed led to a decrease of the tetraploid^{TOM} cell population (Figure 23C).

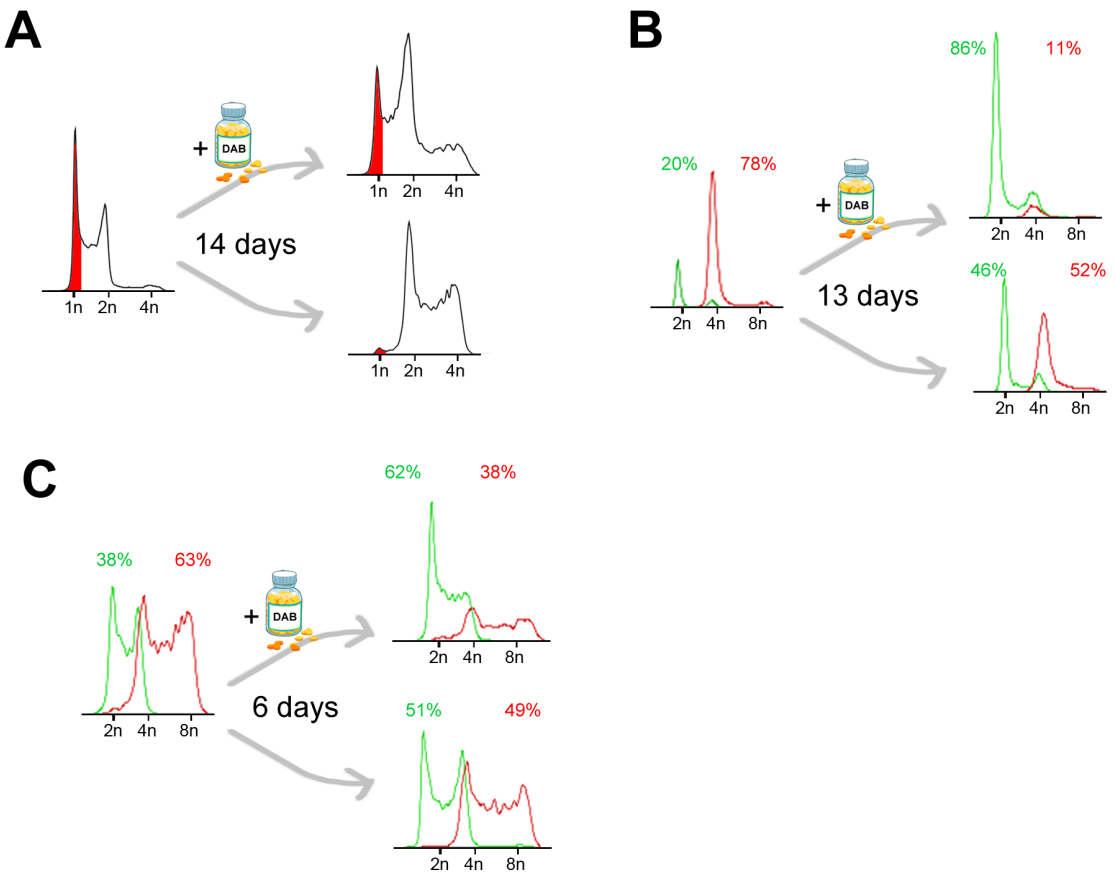


Figure 23: DAB selects for cells with lower ploidy in cell types of different origin. (A) mhaESCs were cultured untreated or treated with 5 µM DAB over 14 days. DNA content staining was performed at day 0 and day 14. G1 haploid cells are labeled in red. Two independent experiments were performed. (B) tdTomato-expressing tetraploid and EGFP-expressing MEFs were mixed in a ratio of 4:1 and cultured untreated or treated with 10 µM DAB for 13 days. DNA content was evaluated by flow cytometry and numbers show the percentage of the individual populations. Three independent experiments were performed but one representative is shown. (C) tdTomato-expressing tetraploid and EGFP-expressing diploid iPSCs were mixed in a ratio of 3:1 and cultured untreated or treated with 1 µM DAB for 6 days. DNA content was evaluated by flow cytometry and numbers show the percentage of the individual populations.

Finally, and based on these results, we tested if DAB could also select for cells with lower ploidy in mixed population of cancer cells. To do this, we took advantage of recently generated diploid and tetraploid clones of the colon cancer cell line DLD1 (Viganó et al., 2018). As earlier

described, we infected the diploid DLD1 cells with lentiviruses encoding for EGFP and the tetraploid DLD1 cells with lentiviruses encoding for tdTomato and mixed them in a 1:9 ratio. Remarkably, a 23-day treatment with DAB led to a dramatic decrease of the tetraploid^{TOM} cell population (Figure 24). Collectively, these results indicate that DAB is able to select for cells with lower ploidy in mixed mammalian cell cultures.

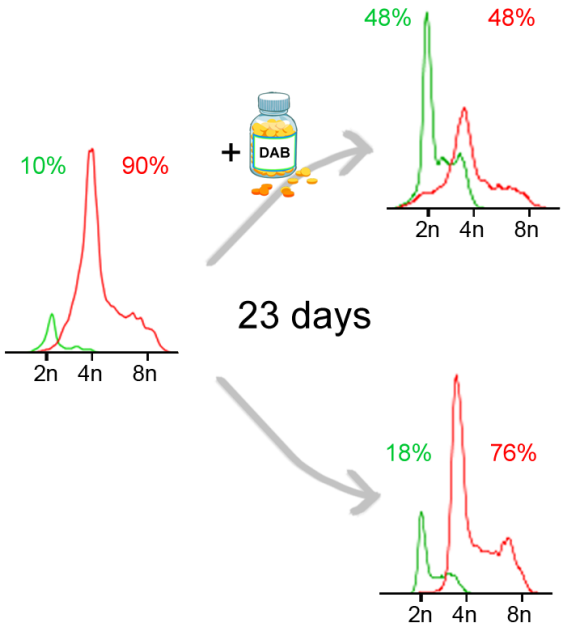


Figure 24: DAB treatment selects for lower ploidy cells in the colon cancer cell line DLD1. tdTomato-expressing diploid and EGFP-expressing tetraploid DLD1 cells were mixed in a ratio 1:9 and cultured untreated or treated with 30 µM DAB for 23 days. DNA content analyses were performed at day 0 and day 23.

3. DAB increases the time in mitosis by activating the SAC

We next sought to investigate the molecular mechanisms driving ploidy selection upon DAB treatment. We first investigated if DAB generated DNA damage, which would be more toxic on cells with more DNA or higher ploidy. To test this, we evaluated the levels of a phosphorylated H2AX (γH2AX), a widely used marker of DNA damage and replication stress, by high-throughput microscopy using the topoisomerase poison Doxorubicin as a positive control (Figure 25A). These experiments did not reveal a major genotoxicity of DAB. Next, we investigated if DAB could perturb DNA replication by measuring the incorporation of the nucleotide analog EdU (5-ethynyl-2'-deoxyiridine) (Figure 25B). However, we also failed to see any significant impact of DAB in hampering DNA replication. Consistent with these results, karyotype analyses failed to reveal any obvious signs of genomic instability such as chromosomal breaks, gains or rearrangements, in mhaESCs treated with DAB for 21 days (Figure 25C, 25D). These results are important since they support the use of DAB as a chemical tool to stabilize the haploid state in mammalian cell cultures.

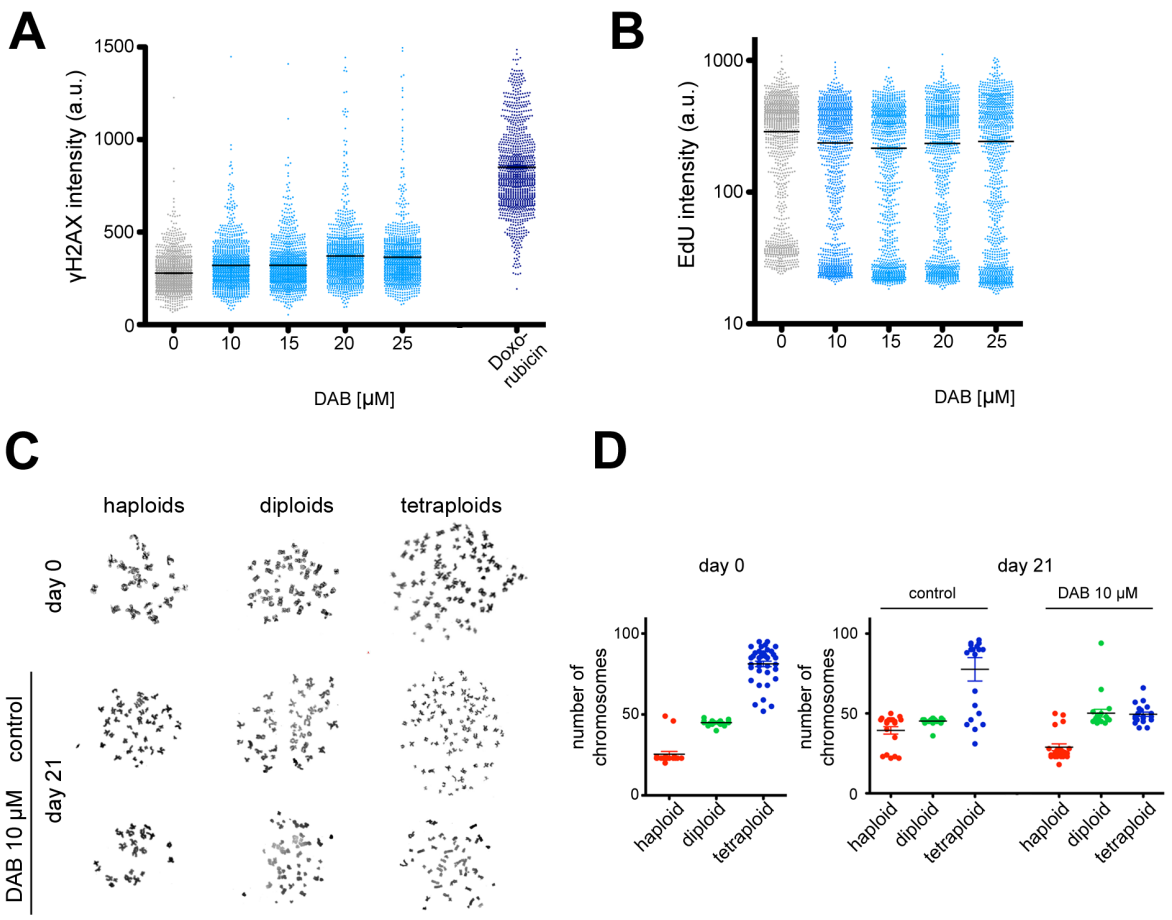


Figure 25: DAB does not induce genomic instability. (A) High throughput microscopy analysis of γH2AX intensity per nucleus in haploid and diploid HAP1 cells treated with increasing doses of DAB for 24 h. Centerline indicates mean values. γH2AX-induction by Doxorubicin was used as control. One experiment is shown but three independent ones were performed. (B) High throughput microscopy analysis of EdU intensity in haploid and diploid HAP1 cells treated with increasing doses of DAB for 24 h followed by 30 min EdU pulse. Centerlines indicate mean values. Three independent experiments were performed but one representative is shown. (C) Representative examples of metaphase spreads from haploid, diploid and tetraploid HAP1 cells at day 0 and day 21 untreated or treated with 10 μM DAB treatment. (D) Average number of chromosomes from the metaphase spreads obtained in (C). 19 metaphases or more per condition were analyzed. Centerline indicates mean values.

After ruling out potential effects of DAB on DNA replication and repair, we explored the potential link of this compound with mitosis. As mentioned above, DAB is a precursor of the microtubule targeting drug Paclitaxel. This, together with the fact that haploid cells suffer from chromosome segregation problems in mitosis, led us to explore if DAB could work somewhat similarly to Paclitaxel in its effects on haploid cells. Supporting our hypothesis, a low dose of Paclitaxel was also able to promote haploidy in a mixed (1:4) culture of haploid and diploid HAP1 cells (Figure 26A). Paclitaxel stabilizes mitotic spindle microtubule polymers by binding to tubulin thus impairing the formation of a properly aligned metaphase plate. This leads to the activation of the spindle activation checkpoint (SAC) with the consequent mitotic lengthening and apoptosis. To assess whether DAB could also activate the SAC we used a U2OS cell line expressing a CyclinB-

mCherry fusion protein, and monitored the time required to degrade cyclin B after mitotic entry (Figure 26B). Cyclin B rises to maximum levels of expression at the nuclear envelope breakdown and is lowest at anaphase onset due to the silencing of the SAC and the consequent activation of APC (that degrades cyclin B). Similar to Nocodazol or Paclitaxel although to a lower extent, DAB increased the timed to achieve CyclinB-mCherry degradation upon mitotic entry, indicating that DAB also activates the SAC, extending the duration of mitosis.

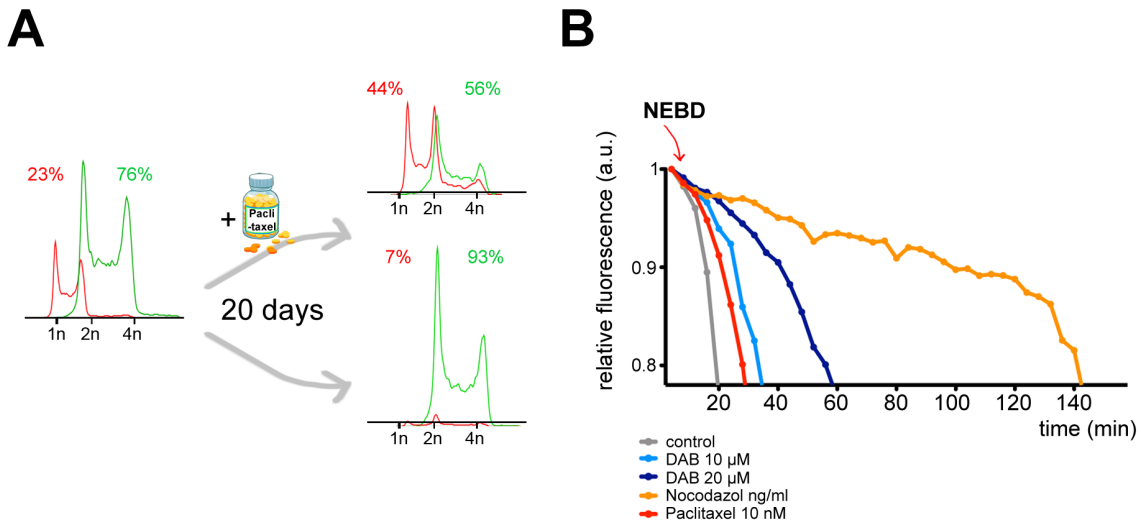


Figure 26: DAB mimics low-dose Paclitaxel treatment. (A) tdTomato-expressing haploid and EGFP-expressing diploid HAP1 cells were mixed in a ratio of 1:4 and cultured untreated or treated with 15 nM Paclitaxel for 20 days. DNA content analyses were evaluated by flow cytometry and numbers indicate the percentage of the individual populations. (B) Graph showing the relative fluorescence levels of CyclinB-mCherry over time upon treatment of the stated compounds in a U2OS cell line expressing CyclinB-mCherry. Cyclin B degradation was monitored by live-cell imaging and levels evaluated from the nuclear envelope breakdown (NEBD) until anaphase. Only cells with apparent cell division were monitored and a minimum of 10 cell divisions was evaluated per condition.

4. DAB activates the SAC in a ploidy-dependent manner

We next asked whether activation of the SAC could explain the ploidy-dependent toxicity observed after treatment with DAB. To test this, we first measured the mitotic index (percentage of cells accumulated in mitosis) in HAP1 cells of different ploidy by analyzing the number of phospho-histone H3 (pH3)-positive cells after DAB treatment (Figure 27A). While untreated haploid, diploid or tetraploid cells showed a similar percentage of mitotic cells, the treatment with DAB led to a ploidy-dependent accumulation of mitotic cells. This observation was recapitulated in mouse ESCs (Figure 27B). Further supporting this view, MAD2 intensity per kinetochore cells was increased in a ploidy-dependent manner in HAP1 cells and further increased upon DAB-treatment (Figure 27C).

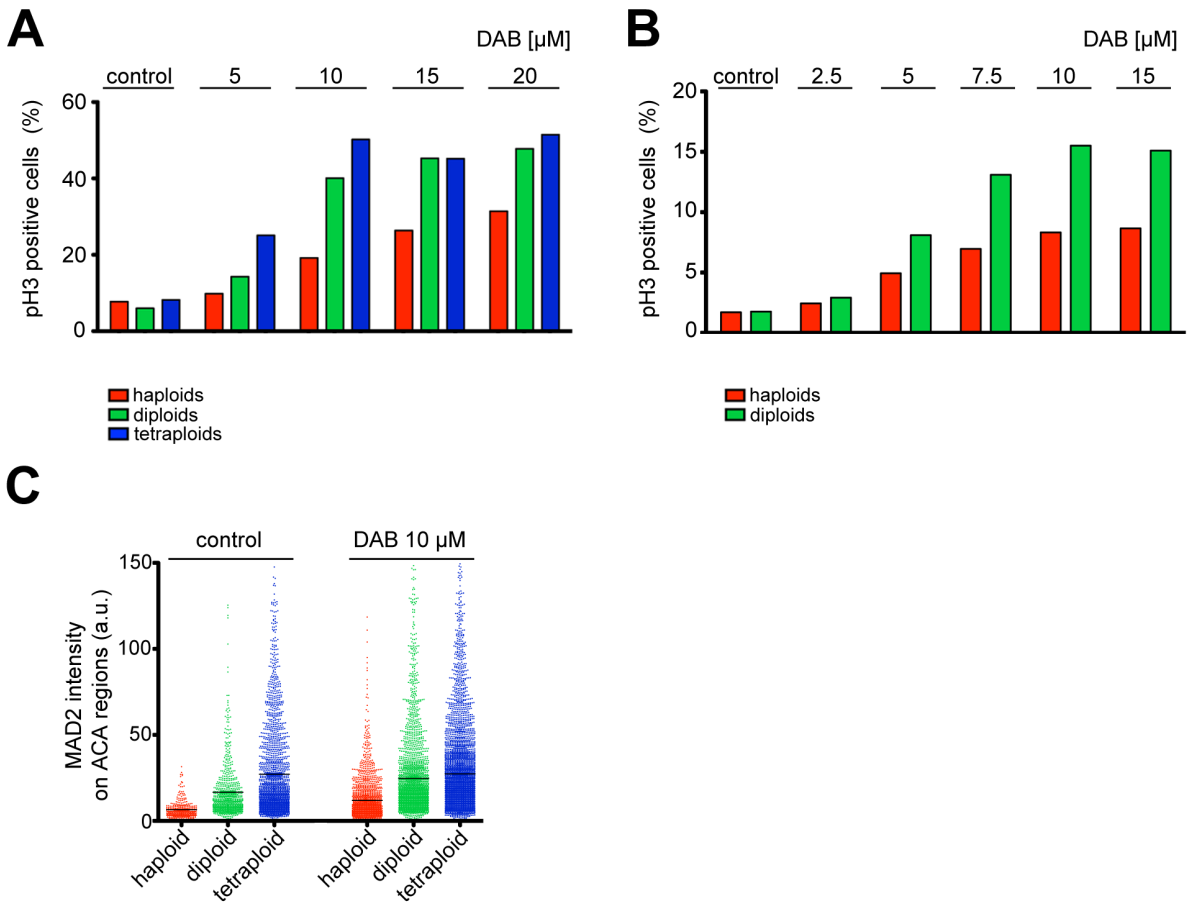


Figure 27: Ploidy-dependent activation of the SAC upon DAB treatment: (A) and (B) High throughput microscopy analysis showing the percentage of phospho-histone 3 (pH3)-positive haploid, diploid and tetraploid HAP1 cells (A) or haploid and diploid mouse ESCs (B) treated with increasing doses of DAB for 16 h (A) or 6 h (B). Two (B) and three (A) independent experiments were performed but one representative is shown. (C) Immunofluorescence staining of MAD2 from HAP1 cells treated with 10 µM DAB o/n. MAD2 intensity on regions colocalizing with ACA (centromeric region) was analyzed in cells in prometaphase (n=5-19 cells).

Finally, haploid, diploid and tetraploid HAP1 cells were infected with lentiviruses expressing a fusion protein between the Histone 2B and RFP (H2B-RFP) and used to perform live-cell video recording to monitor mitotic dynamics and overall cell cycle progression upon DAB treatment. This experiment allowed us to quantify the time spent by each cell in interphase and mitosis. In addition, we could estimate the SAC-dependent (red; from NEBD to metaphase plate formation) and SAC-independent (green; from metaphase plate formation to cytokinesis) times of each mitosis. These analyses revealed several interesting observations: First, SAC-dependent, but not SAC-independent times increase in a ploidy-dependent manner following DAB incubation (Figure 28A, B, C), further revealing that DAB activates the SAC. Second, the treatment with DAB was toxic in a ploidy-dependent manner. Third, upon DAB treatment most tetraploid cells (and some diploids) remain trapped in metaphase due to a constitutive activation of the SAC, ultimately leading to cell death. In contrast, most haploid cells can complete a successful mitosis even in the presence of DAB (Figure 28A).

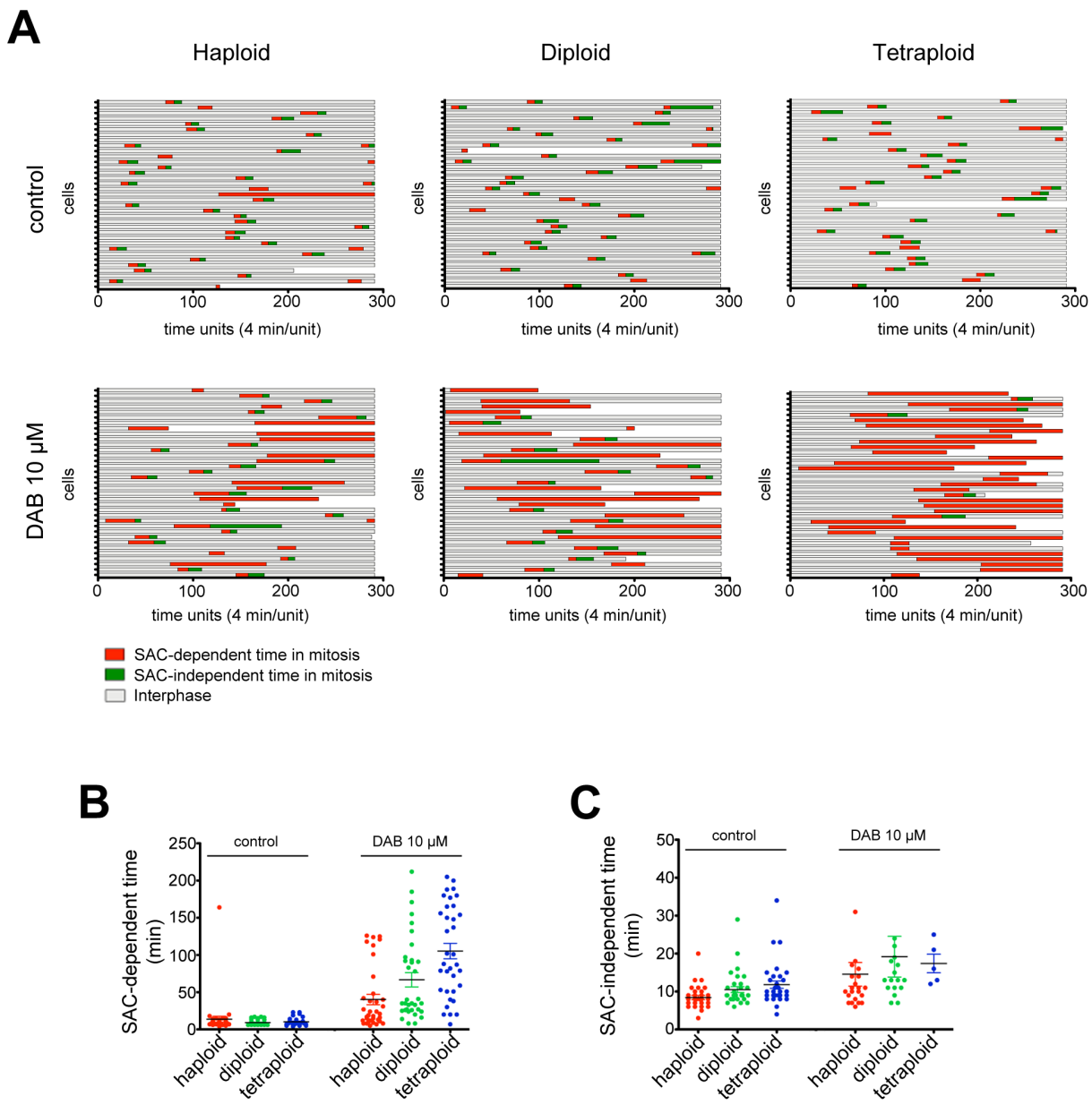


Figure 28: SAC-dependent time increased upon DAB treatment in a ploidy dependent-manner. (A) Schematic representation of time spent in mitosis by individual, RFP-H2B expressing, haploid, diploid and tetraploid HAP1 cells untreated or treated with 10 µM DAB. Light gray bars showed the time in interphase, red bars showed the SAC-dependent time in mitosis and green bars showed the SAC-independent time in mitosis. SAC-dependent time was defined as the time between chromosome condensation and metaphase plate observation. SAC-independent time was defined as the time between metaphase plate observation and cytokinesis. Cells were monitored every 4 min for a total of 16 h and a minimum of 30 cells per condition was analyzed. (B) and (C) Graphs showing SAC-dependent (B) and SAC-independent time (C) in haploid, diploid and tetraploid HAP1 cells quantified from (A).

Given that Paclitaxel is a microtubule-targeting drug, we asked if DAB also has an impact on spindle morphology. To evaluate this, haploid, diploid and tetraploid HAP1 cells were treated with DAB and mitosis were analyzed by confocal microscopy (Figure 29A). Consistent with the previous experiments, all tetraploid and most diploid HAP1 cells treated with DAB were trapped in prometaphase, whereas cells in anaphase/telophase could be easily observed in DAB-treated haploid HAP1 cells. Moreover, DAB treated diploid and tetraploid HAP1 cells showed a significant

increase of spindle aberrancies in prometaphase compared to haploid HAP1 cells (Figure 29B, 29C).

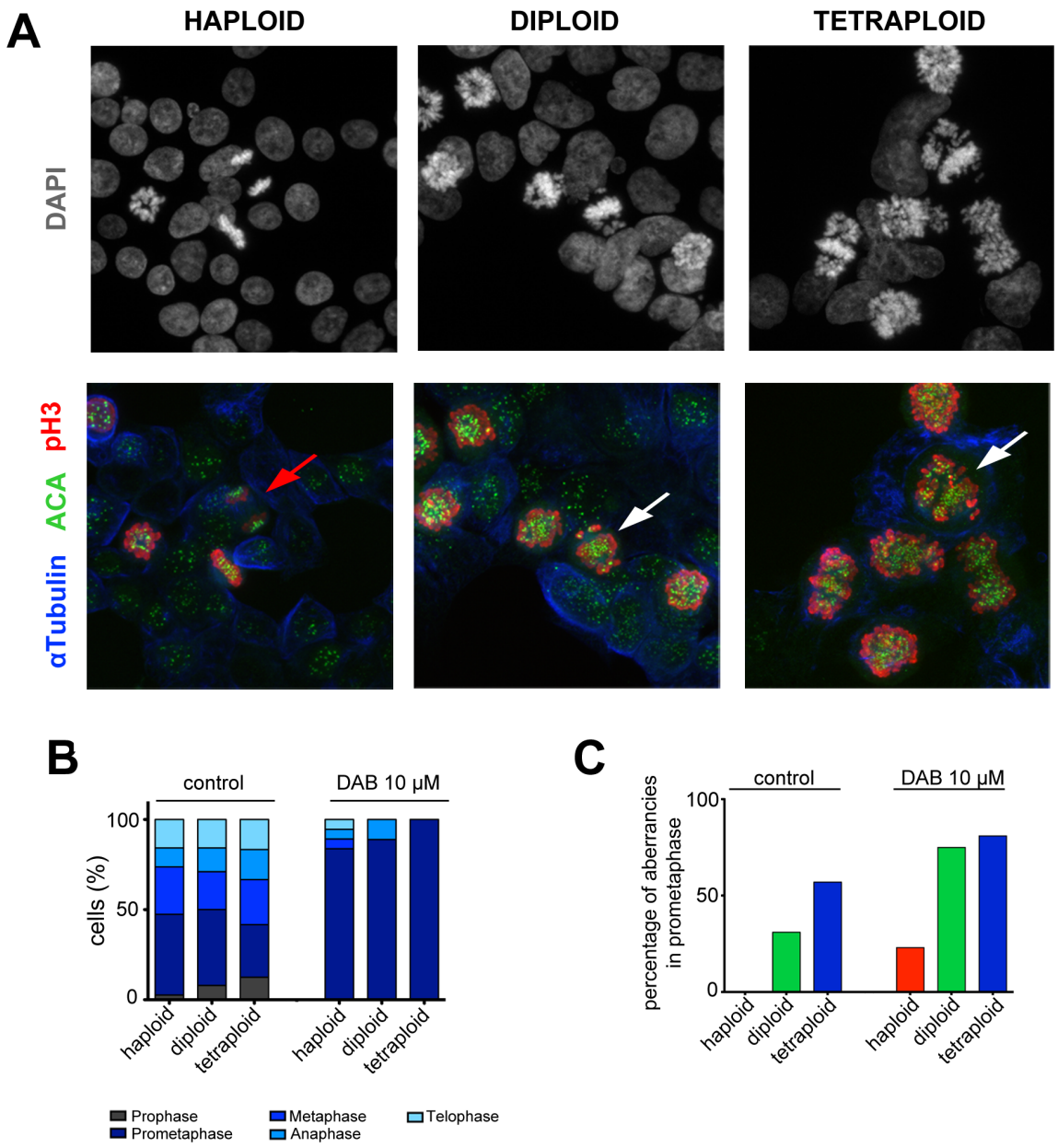


Figure 29: Prometaphase spindle defects induced by DAB treatment. (A) Haploid, diploid and tetraploid HAP1 cells were treated with 10 µM DAB for 16 h and processed for immunofluorescence staining. Representative immunofluorescence staining is shown. DNA was stained with DAPI (grey), αTubulin in blue, anti-centromeric antibody staining in green and pH3 in red. Red arrows show a haploid cell in anaphase, white arrows indicating aberrant prometaphases in diploid and tetraploid cells. (B) Quantification of immunofluorescence staining from A shows the percentage of cells in the different phases of mitosis. A minimum of 20 mitosis per condition were evaluated. (C) Percentage of aberrant prometaphase spindles from the cells in (B). An aberrant prometaphase spindle was defined as spindle with lagging chromosomes and/or aberrant tubulin.

All taken together, our results indicate that DAB is a weak SAC activator, likely working by slightly perturbing microtubule function. Whereas haploid cells, due to their lower ploidy, might be able to complete the alignment of mitotic chromosomes in the metaphase plate even in the presence of DAB, diploid, and even more, tetraploid, cells show a significant impairment of mitotic progression by this drug ultimately leading to cell lethality. Moreover, the fact that our results are recapitulated with low-dose Paclitaxel reveal, for the first time, that a slight activation of the SAC can select for cells with lower ploidy in mixed populations of mammalian cells.

DISCUSSION

1. Evidences for a ploidy checkpoint in mammalian cells

In one of the first manuscripts reporting the derivation of haploid cells from frogs, Freed described the phenomena of "diploidization" with the following words: "*Study of the chromosomes of dividing cells in the cultures shows that a minority of diploid cells (five per cent or less) occur in the early subculture generations. These increase, with succeeding transfers, relative to the haploid population, until eventually they become the majority type. Such diploidized cultures of haploid origin do not, however, grow at the same rate as do the cultures of normal diploid origin. This is taken to suggest the origin of the diploidized cells by an endomitotic process"* (Freed, 1962). Similar observations were further described in haploid cells derived from *Drosophila*, the near-haploid human cancer cell line KBM7 and in haploid ESC lines isolated from different animals (Debec, 1984; Kotecki et al., 1999; Martin Leeb & Wutz, 2012; W. Li et al., 2014; Sagi et al., 2016; Yilmaz et al., 2016). In agreement with these published reports, we also observed a rapid increase of diploid cells in the human cancer cell line HAP1 as well as in newly generated mhaESCs upon culturing suggesting the existence of a true conversion of haploid into diploid cells. However, contrary to the established view of "diploidization", we observed that this is a rare event. By performing competition assays between haploid and diploid cells, we detected a reduced fitness in the haploid population compared to their diploid counterparts (Figure 9A, 9B). The ability of diploid cells to outcompete the growth of the haploids seems to be a general phenomena among all reported haploid cell cultures (Debec, 1984; Kotecki et al., 1999; Martin Leeb & Wutz, 2012; W. Li et al., 2014; Sagi et al., 2016; Yilmaz et al., 2016). Yet, interestingly, this does not seem to be the case for haploid cells isolated from the fish *Danio rerio* or the monkey *Macaca fascicularis* (Yang et al., 2013; Yi et al., 2009). Haploid monkey and zebrafish ESC lines were stable without further manipulation in their haploid status for up to 140 days and 500 days of culture, respectively. However, the reasons that enable stable haploid ESCs in these animals remain unknown.

Competition experiments led us to hypothesize that single-cell sorted haploid cells should remain haploid. Indeed, we have here demonstrated that this is the case, not only for HAP1 cells but also for the unstable mhaESCs (Figure 9C, 12F). Of note, in these haploid cultures we detected a small, but progressive, emergence of diploid cells, which could only be explained by real diploidization since these cell lines originate from a single haploid cell. Although diploidization is a very rare event, once diploid cells originate they progressively take over the culture due to their better proliferation capacities. While the underlying mechanism of diploidization in haploid mammalian cells is unknown, we speculate that it might be similar to what occurs during tetraploidization of diploid cells. In this regard, mitotic nondisjunction by furrow regression leads to cytokinesis failure and is a common mechanism to generate tetraploid cells. In fact, nondisjunction was previously suggested to explain the diploidization of KBM7 (Bürckstümmer et al., 2013), although there is no data to support this idea. Besides mitotic non-disjunction, endoreplication

(cells replicate the genome repeatedly skipping mitosis), endomitosis (skipping cytokinesis), cell fusion or mitotic slippage (a prolonged activation of the SAC leads to the exhaustion of cyclin B levels so that cells exit mitosis without anaphase or cytokinesis) have been reported as sources of tetraploidy. While formal proof is still lacking, based on our findings of prolonged mitosis and chromosome mis-segregation, mitotic slippage or non-disjunction seem as plausible candidates of the diploidization of mammalian haploid cells.

In what regards to the reasons behind the reduced fitness of mammalian haploid cells, this is explained by the activation of a P53-dependent cytotoxic response that limits their viability. In turn, P53 activation is due to chromosome segregation defects including lagging chromosomes, anaphase bridges and micronuclei explaining. Consistent with the presence of a P53-dependent haplopidy checkpoint, P53 deletion rescued the viability of mhaESCs and helped to stabilize haploidy in culture (Figure 12D, 12E). Interestingly, numerous studies in tetraploid and aneuploid cells have revealed that these cells are also prone to accumulate chromosome segregation defects during mitosis, and their growth is also limited by the activation of P53 (Andreassen et al., 2001; Ganem et al., 2014; Ganem & Pellman, 2007; M. Li et al., 2010; Thompson & Compton, 2010). Given the similarity of these phenomena, we here wish to suggest the existence of a general "ploidy checkpoint", which limits the expansion of cells with an altered ploidy by P53-dependent cytotoxic or cytostatic responses (Figure 30).

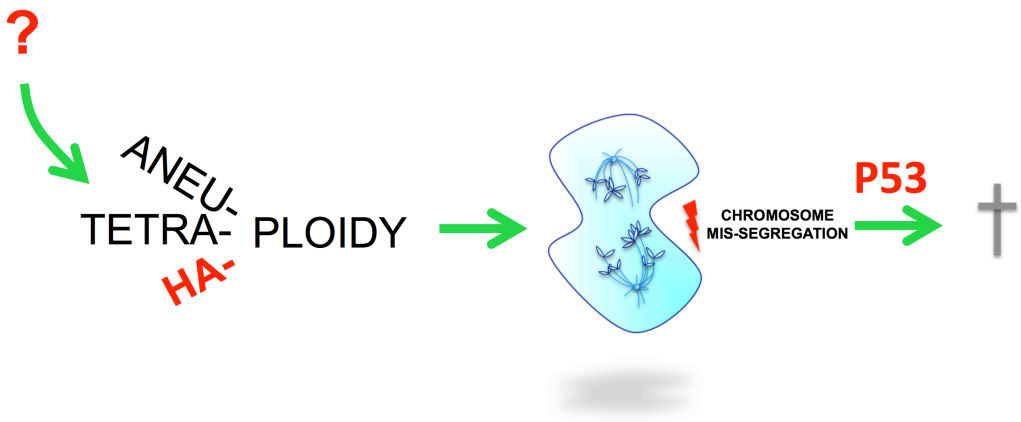


Figure 30: A P53-dependent ploidy checkpoint in mammalian cells. A non-euploid mammalian cell detects by unknown mechanism the chromosome number aberrancies. These cells accumulate chromosome mis-segregation defects leading to a P53-dependent cytotoxic reponse.

How haploidy, or aneuploidy and tetraploidy for that matter, leads to P53 activation is not fully understood. In yeast, cell size is controlled by Pom1, a cell-division inhibitory factor is diluted as cells grow in size (Martin & Berthelot-Grosjean, 2009; Moseley, Mayeux, Paoletti, & Nurse, 2009). While there seems to be no cell-size control in more complex pluricellular organisms (Leslie, 2011), organ size is regulated by the Hippo pathway, which for instance regulates cell numbers in postnatal livers (Lloyd, 2013). Interestingly, the group of David Pellman showed that the Hippo

pathway could be responsible for the activation of P53 in mammalian tetraploid cells, through a pathway involving the LATS2 kinase (Ganem et al., 2014). However, we could not detect activation of the Hippo pathway in mammalian haploid cells. While these results were not incorporated into this thesis, we also performed quantitative mass spectrometry and microarray in haploid and diploid mammalian cells, trying to identify imbalances that could explain the reduced fitness in haploid cells. Unfortunately, and consistent with previous transcriptomic data (Martin Leeb & Wutz, 2012), none of these approached revealed major differences that could explain our observations. Nevertheless, given the above mentioned similarities between haploid, aneuploid and tetraploid cells, it could simply be that the mitotic spindle has evolved to deal with a specific amount of DNA/chromosomes, and that any deviation from the natural amount affects chromosome segregation, ultimately leading to genome instability and the activation of P53. Our data revealing chromosome segregation problems, and a noisy arrangement of chromosomes at the spindle in mammalian haploid cells supports this view (Figure 15, 18).

In summary, we here propose the existence of a ploidy checkpoint that is responsible for the gradual loss of haploidy that is observed in mammalian haploid cell cultures. Based on our discovery, we have been able to define two strategies (P53-deficiency or single cell sorting), capable of stabilizing the haploid state in culture. Nevertheless, P53-deficiency will stabilize haploidy at the extent of genomic instability, so that we favor single-cell sorting. These simple procedures should significantly facilitate future work with mammalian haploid cells.

As mentioned above, while mammalian haploid cell lines have been generated, differentiation seems to significantly accelerate diploidization rates, and nobody has yet succeeded in generating mammalian haploid tissue. For instance, in the original study reporting the derivation of mhaESCs, it was shown that these could contribute to adult tissue when aggregated to WT diploid ESCs, but this tissue was made of diploid cells (Martin Leeb & Wutz, 2012). A follow-up study reported that a haploid karyotype was incompatible with postgastrulation in chimeric embryos with high contribution from the haploid cells (M. Leeb et al., 2012). Based on our finding that P53-stabilizes haploidy *in vitro*, we immediately asked whether this genetic condition would also enable the generation of differentiated haploid mouse tissue. Previous work had shown that while tetraploid ESCs also activate P53-dependent apoptosis, P53-deletion enabled the generation of late-stage mouse tetraploid embryos (Horii et al., 2015). In addition, there have been reports of liveborn tetraploid human infants (Golbus, Bachman, Wiltse, & Hall, 1976; Horii et al., 2015; Pitt et al., 1981; Shiono, Azumi, Fujiwara, Yamazaki, & Kikuchi, 1988), such that it seemed plausible that the generation of a haploid mammal, or at least embryo, could be achieved. Besides its general interest in developmental biology, the access to mammalian haploid tissue could significantly facilitate forward genetic screenings *in vivo*.

We initially started by trying to differentiate P53KO mhaESCs in vitro, with the aim to generate a primary differentiated haploid cell line that we could use for further studies such as genetic screens. However, while the cells differentiated normally, they invariably became diploid. Besides this in vitro work, we also tried to generate haploid mammalian tissue. To do so, and trying to maximize the contribution of haploid cells to a specific organ, we used mice carrying a mutation in the tyrosine catabolic enzyme fumarylacetoacetate hydrolase (FAH) that is deleterious for liver cells (Azuma et al., 2007; Espejel et al., 2010). The viability of these mice can be rescued by two means: 1) by the constant supply of the drug NTBC or 2) by microinjecting wild type stem cells (e.g. iPSCs) into FAH-depleted blastocysts, which leads to viable chimeric mice where most of the liver is derived from functional hepatocytes differentiated from the wild type cells (Espejel et al., 2010). We hypothesized that the microinjection of *P53*$^{-}$; *KFP*^{T} mhaESCs into FAH-deficient blastocysts could lead to viable mice with livers made to a large extent from mhaESCs-derived cells. We also chose to focus on the liver since it is rather tolerant to genomic instability and/or ploidy alterations, as exemplified by the fact that polyploidy gradually increases with age in the mammalian liver (Gentric & Desdouets, 2014; Medvedev, 1986). While the embryos we obtained from these experiments did not show a preferential accumulation of the mhaESCs-derived cells in their livers, which we believe is due to the maternal contribution of FAH, we did obtain embryos with significant contribution of the P53-deficient mhaESCs in various organs, which we could identify on the basis of Katushka expression (Figure 19 B, 19C). We have promising preliminary data based on centromeric FISH intensities, which will be consistent with the presence of haploid cells in certain tissues such as the dermis or the brain in these embryos (Figure 20). While we are currently performing additional experiments to formally proof that these cells are indeed haploid, it might well be that we have indeed succeeded in making mammalian tissue containing haploid cells.

2. Chemical stabilization of haploidy

Given the poor stability of the haploid state in mammalian cell lines, it would be highly desirable to have compounds capable of overcoming this limitation. Under this premise, we here carried out a chemical screening to identify compounds that either select for haploid cells or diploid cells in a mixed culture. From a total of 978 compounds, six compounds were identified (and validated) to stabilize haploidy, and 5 to accelerate the loss of haploid cells from these cultures (Figure 21C, 21D).

The five compounds favoring the diploid condition identified in our screen belong to the group of statins, a widely used class of lipid-lowering drugs. Statins are inhibitors of the HMG-CoA reductase, the rate-limiting step in the mevalonate pathway, playing a central role in

cholesterol synthesis. Thus, these compounds are generally used to decrease serum cholesterol levels and thus to prevent cardiovascular disease (Demierre, Higgins, Gruber, Hawk, & Lippman, 2005). At the cellular level, the mevalonate pathway is crucial for maintaining the structure and function of cell membranes. Interestingly, statins have been recently identified to increase cell size in a drug screening aiming to find compounds regulating cell size control (Miettinen & Björklund, 2015). In fact, it was shown that statins increase the overall duration of the cell cycle with the consequent slowdown in proliferation and increased cell size. In addition, statins also increase the cellular protein content through autophagy mediated by the mevalonate pathway. It is tempting to speculate that haploid cells could display a misbalance between cell size, membrane area and protein content compared to the diploid cells. Indeed, we observed that haploid cells do not have half of the size or half of the protein content compared to their corresponding diploid cells. Thus, additional interference into cell size control by using statins could lead to a selective disadvantage for the haploids upon cell culture. As an alternative possibility, the reduction of proliferation after statin treatment in cells, could selectively affect haploid cells to a greater extent as they have already intrinsic poor growth capabilities (as described above). Of note, HMG-CoA-reductase inhibition with statins has been shown to be rather pleiotropic, leading to anti-inflammatory, immunomodulatory, anti-angiogenetic and pro-apoptotic effects. Hence, we cannot rule out that the effect of statins on ploidy is independent of the HMG-CoA-reductase. However, and while we tried to address this experimentally, the essentiality of this enzyme precluded us to formally test this possibility by generating knockouts in haploid cells.

Of note, lipid metabolism might play a more general role in cells with an altered ploidy. In a recent study, the group of Angelika Amon performed an unbiased drug screening aiming for the discovery of drugs selectively killing aneuploid cells. In such a screen, they found that inhibition of the UDP glucose ceramide glycosyltransferase, an essential enzyme in the sphingolipid homeostasis, induces apoptosis in aneuploid cells via increased ceramide levels (Tang et al., 2017). In addition, they report that aneuploid cells display elevated levels of ceramides and therefore harbor an intrinsically dysregulated sphingolipid metabolism. Although the sphingolipid pathway and the mevalonate pathway do not interplay directly with each other, they are tightly intertwined and cross talk between these pathway occurs (Gulati, Liu, Munkacsi, Wilcox, & Sturley, 2010). Taken together with our work, we could speculate about the importance of lipid biosynthesis for mammalian cells with an altered ploidy such as haploid cells.

In regards to the six compounds that facilitate the maintenance of haploid cells, we focused on studying in detail the mechanism of action of DAB, the top hit of our screening. DAB is a chemical compound isolated from european yew trees and used as precursor in the synthesis of Paclitaxel (B. J. Li et al., 2017; Malik et al., 2011). Based on the chemical similarity between DAB

and Paclitaxel, we speculated that the mechanism by which DAB favors haploidy could resemble what Paclitaxel does on cells. In fact, we observed that a low-dose treatment of Paclitaxel also selects for haploid cells in a culture mix with diploids (Figure 26A). Moreover, either DAB also selected for cells with lower ploidy in mixed cultures of haploid/diploid/tetraploid mammalian cells, revealing that SAC activation provides one way to selectively kill cells with a higher number of chromosomes. Although we cannot be certain that the target of DAB is tubulin, we have clearly shown that DAB, similar to Paclitaxel, activates the SAC and elongates mitosis (Figure 26B, 27, 28). These results demonstrated that interference with the mitotic process is an effective method to select haploid cells. Along these lines, recent studies have revealed that WEE1 (Takahashi et al., 2014) or CDK1 inhibition (He et al., 2017) could facilitate the maintenance of haploid cells *in vitro,* which however is somewhat surprising given that these two enzymes have opposing roles in regulating mitotic entry. Moreover, we should mention that neither of these inhibitors had a significant effect on stabilizing haploidy in our hands, though we cannot rule out that different experimental conditions could influence the outcome of the experiments.

Regardless of these differences, it seems clear that mitosis problems lie at the core of the reduced fitness of mammalian haploid cells and as such, treatments directed to alter mitosis might offer a solution to this problem. But, how would an activated SAC select for cells in a ploidy dependent manner? We speculate that cells with a higher ploidy need more time in mitosis to properly attach and bi-orientate all chromosomes to the kinetochores before proceeding to the anaphase. In other words, it is intuitive to think that the more chromosomes the longer time a cell needs to organize the metaphase plate and proceed through mitosis. If microtubules are challenged, this would further aggravate the situation and the effect would again be proportional to the number of chromosomes that need to be properly aligned. Accordingly, when cells are treated with DAB, the SAC-dependent time increases in a ploidy dependent manner (Figure 31). Supporting this general idea, the SAC is not essential in organisms carrying few chromosomes such as *Drosophila melanogaster* (4 chromosomes) or *Caneorhabditis elegans* (6 chromosomes), likely since they can arrange their chromosomes at the metaphase plate very fast (Kitagawa, 2009; Orr, Bousbaa, & Sunke, 2006). Moreover, and to our surprise, MAD2, an essential component of the SAC, is not essential in the HAP1 cell line (Blomen et al., 2015) (Raaijmakers et al., 2018). However, HAP1 cells derive from a cancer cell line and have been subjected to multiple selections, so that it is possible that they have selected for mutations that enable their growth in the haploid state.

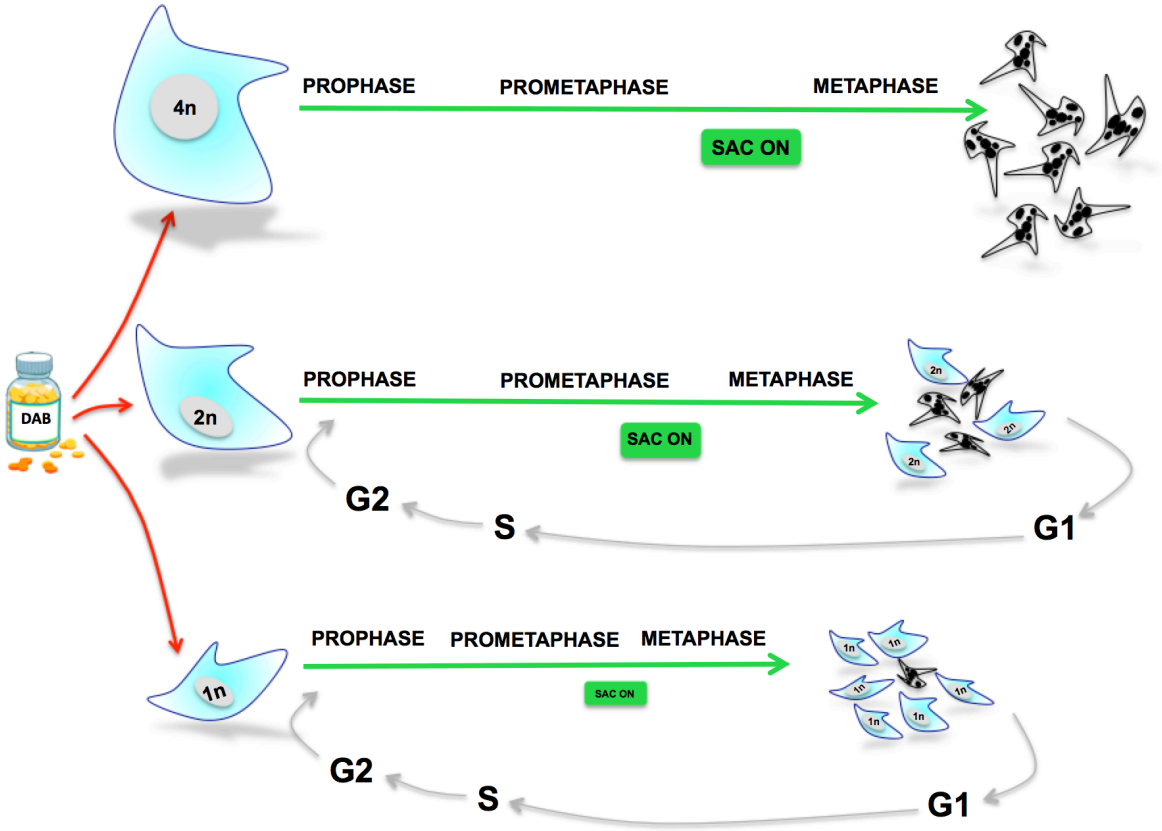


Figure 31: Ploidy-dependent effect upon DAB treatment. DAB increases the SAC-dependent time in a ploidy-dependent manner. In haploid cells the SAC-dependent time is overall increased upon DAB treatment but short enough to allow most of the cells to exit from mitosis. However, with increasing ploidy, the SAC-dependent time under treatment increases and the exit of mitosis becomes precluded.

In support to the concept that SAC-activating drugs can be preferentially toxic for cells with more chromosomes, the SAC has been shown to work as a rheostat rather than an all-or-nothing checkpoint (Collin et al., 2013). In fact, the strength of SAC activation depends on the number of signaling centers and critically on the amount of MAD2 recruited to kinetochores. Consistent with this, kinetochore-attached MAD2 levels correlated with the number of chromosomes in our experiments and were further increased upon DAB treatment in a ploidy-dependent manner (Figure 27C). This reinforced the idea that DAB allowed the survival of cells with lower ploidy due to their reduced time required to organize the metaphase plate. It remains to be tested whether other SAC-activating drugs, such as Nocodazole (inhibits reversibly the microtubule polymerization) or Monastrol (inhibits mitotic kinesin Eg5), can also select cells in a ploidy-dependent manner. However, these compounds have a more profound impact on microtubules than Paclitaxel and they might indistinctly affect haploid or diploid cells.

We are currently investigating whether the use of DAB can help the generation of mhaESCs, or the development of mammalian haploid tissue. Nevertheless, and besides its usefulness to stabilize

the haploid state, the capacity of DAB to select for cells with lower ploidy in mixed cultures offers interesting possibilities. For instance, we are currently exploring if DAB can be used to selectively eliminate aneuploid cells containing 1 additional chromosome from mixed cultures. If this holds, it would be interesting to test if DAB could be in general used as a way to select for euploid cells in various cultures (e.g. ESCs). In addition, and while to our knowledge DAB has not been used medically, given the high prevalence of aneuploid or polypoid cells in cancer, DAB or lower doses of Paclitaxel could have potential in selectively killing these cells *in vivo.*

In addition to DAB, we identified five more compounds that stabilize the haploid state. In contrast to the compounds that favor diploidy, which all belong to the class of statins, there was seemingly no common mechanism of action within this group. The two most potent compounds stabilizing haploidy besides DAB were Nilotinib, a kinase inhibitor that inhibits, among others, the cABL tyrosine kinase and which is used in the treatment of chronic myeloid leukemia (Wyse et al., 2016) and Diclazuril, an antibiotic used in the prevention of bovine coccidiosis for which no known target exists (Zechner et al., 2015). Regardless of their assumed mechanism of action, we cannot rule out that the effect of these compounds on haploid cells is not due to an unknown target. For this reason, we are currently investigating if, similarly to DAB, they also impact mitotic progression or can trigger the activation of the SAC.

CONCLUSIONS

PART I: Defining a novel (ha)ploidy checkpoint

1. A competitive growth disadvantage, rather than diploidization, is responsible for the progressive loss of haploidy in mammalian cell lines. Consistently, single-cell sorting facilitates the long-term maintenance of haploidy in HAP1 cells and mhaESCs.

2. While diploidization (e.g. the conversion of a haploid into a diploid cell) does happen in haploid mammalian cells, this is a rare event. However, once diploid cells emerge they progressively overgrow haploids.

3. The reduced fitness of mhaESCs is associated to chromosomal segregation defects leading to cell death within or shortly after mitosis.

4. A P53-dependent cytotoxic response limits the viability of haploid HAP1 cells and mhaESCs.

5. We suggest the existence of a general "ploidy checkpoint", which integrates observations made in haploid, aneuploid and tetraploid mammalian cells, by which cells with an altered ploidy frequently suffer from chromosome mis-segregation, leading to genomic instability, P53 activation and ultimately cell cycle arrest or apoptosis.

6. When aggregated to diploid WT ESCs, P53-deficient mhaESCs contribute to chimeric embryos.

7. Preliminary evidence from these experiments supports that certain embryonic organs such as skin, might contain haploid P53-deficient cells.

PART II: Chemical stabilization of haploidy in mammalian cells

1. A chemical screen led to the identification of several compounds that favor either haploid or diploid cells in mixed cultures.

2. All identified compounds that favor the growth of diploid cells belong to the group of statins, drugs used to lower cholesterol levels.

3. DAB, a precursor of Paclitaxel, was the top hit in the class of compounds favoring the growth of haploid cells.

4. DAB increases the time in mitosis by activating the SAC.

5. DAB facilitates the long-term maintenance of haploidy in HAP1 cells and mhaESCs.

6. DAB and low-dose Paclitaxel, select for cells with a lower ploidy in mixed cultures of haploid, diploid and tetraploid cells.

CONCLUSIONES

PART I: Definiendo un nuevo “punto de control” basado en la (ha)ploidia cellular

1. Una desventaja en el crecimiento competitivo, en lugar de diploidización, es la responsable de la pérdida progresiva de haploidía en líneas celulares de mamífero. De forma consistente, la separación celular mediante técnicas de citometría de flujo de células individuales facilita el mantenimiento a largo plazo de la haploidía en células HAP1 y mhaESCs.

2. Mientras que la verdadera diploidización (es decir, la conversión de una célula haploide en una diploide) ocurre, es un evento raro. Sin embargo, una vez que las células diploides aparecen en el cultivo, crecen rápidamente y desplazan a las haploides.

3. La viabilidad reducida de las mhaESCs está asociada a defectos de segregación cromosómica que llevan a la muerte durante o poco después de la mitosis.

4. Una respuesta citotóxica dependiente de p53 limita la viabilidad de las células HAP1 y mhaESCs.

5. Sugerimos la existencia de un "punto de control” basado en la ploidía celular, que integra observaciones realizadas en células de mamífero haploides, aneuploides y tetraploides, en el que las células con un estado de ploidía alterada sufren con frecuencia una mala segregación cromosómica que lleva a inestabilidad genómica, activación de P53 y, finalmente, parada del ciclo celular o apoptosis.

6. Células mhaESCs deficientes en p53 contribuyen para formar embriones quiméricos cuando son agregadas junto a células ESCs salvajes diploides.

7. Resultados preliminares de estos experimentos sugieren que ciertos órganos embrionarios, como la piel, pueden contener células haploides deficientes en p53.

PART II: Estabilización química de la haploidía en células de mamífero

1. Un análisis masivo de compuestos químicos llevó a la identificación de varios compuestos que favorecen el crecimiento de células haploides o diploides en cultivos mixtos.

2. Todos los compuestos identificados que favorecen el crecimiento de células diploides pertenecen al grupo de las estatinas, compuestos usados para disminuir los niveles de cholesterol.

3. DAB, un precursor del Paclitaxel, es el mejor dentro de la clase de los compuestos que favorecen el crecimiento de células haploides.

4. DAB aumenta el tiempo en mitosis mediante la activación del SAC

5. DAB facilita el mantenimiento de la haploidía a largo plazo de las células HAP1 y mhaESCs.

6. DAB, así como el tratamiento con bajas dosis de Paclitaxel, selecciona las células con menor ploidía in cultivos mixtos de células haploides, diploides y tetraploides.

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ANNEX

Table 2: FDA approved drug-screening library (Z145127, Selleckchem)

COMPOUND	INDICATION	TARGETS	BRIEF DESCRIPTION
(+,-)-Octopamine HCl	Immunology	Others	Octopamine (OA), a biogenic monoamine structurally related to noradrenaline, acts as a neurohormone, a neuromodulator and a neurotransmitter in invertebrates.
1-Hexadecanol		Others	1-Hexadecanol is a fatty alcohol used to make other chemicals.
10-Deacetylbaccatin-III		Others	10-Deacetylbaccatin-III is an antineoplastic agent and an anti-cancer intermediate.
2-Methoxyestradiol	Cancer	HIF	2-methoxyestradiol (2ME2) is a natural metabolite of estrogen that is known to inhibit HIF-1 alpha with an IC50 of 0.71 ± 0.11 μM for the inhibition of BPAEC migration.
2-Thiouracil	Endocrinology	Others	2-Thiouracil is a thiolated uracil derivative that is a known antihyperthyroid agent.
5-Aminolevulinic acid hydrochloride	Neurological Disease	Others	5-Aminolevulinic acid is an intermediate in heme biosynthesis in the body and the universal precursor of tetrapyrroles.
9-Aminoacridine		Others	
Abitrexate (Methotrexate)	Cancer	DHFR	Abitrexate(Methotrexate) is an antimetabolite and antifolate agent with antineoplastic and immunosuppressant activities.
Acadesine	Cardiovascular Disease	AMPK	Acadesine is an AMP-activated protein kinase activator which is used for the treatment of acute lymphoblastic leukemia and may have applications in treating other disorders such as diabetes.
Acarbose	Metabolic Disease	Others	Acarbose is an anti-diabetic drug used to treat type 2 diabetes mellitus and, in some countries, prediabetes.
Acebutolol HCl	Neurological Disease	Adrenergic Receptor	Acebutolol is a β-adrenergic receptors antagonist used in the treatment of hypertension, angina pectoris and cardiac arrhythmias.
Aceclidine HCl		Others	
Acemetacin (Emflex)	Infection	COX	Acemetacin (Emflex) is a non-steroidal anti-inflammatory drug and a glycolic acid ester of indometacin that is a cyclooxygenase inhibitor.
Acetanilide (Antifebrin)	Neurological Disease	Others	Acetanilide is an aniline derivative and has possess analgesic.
Acetarsone		Others	

COMPOUND	INDICATION	TARGETS	BRIEF DESCRIPTION
Acetylcholine chloride	Neurological Disease	AChR	The chemical compound acetylcholine (ACh) is a neurotransmitter in both the peripheral nervous system (PNS) and central nervous system (CNS) in many organisms including humans.
Acetylcysteine	Respiratory Disease	Others	Acetylcysteine is a pharmaceutical drug and nutritional supplement used primarily as a mucolytic agent and in the management of paracetamol (acetaminophen) overdose.
Acipimox	Cardiovascular Disease	Others	Acipimox is a niacin derivative used as a hypolipidemic agent.
Acitretin	Infection	Others	Acitretin is a second generation retinoid used for psoriasis.
Aclidinium Bromide	Neurological Disease	AChR	Aclidinium Bromide inhibits human muscarinic AChR M1, M2, M3, M4 and M5 with Ki of 0.1 nM, 0.14 nM, 0.14 nM, 0.21 nM and 0.16 nM, respectively.
Acyclovir (Aciclovir)	Infection	Others	Acyclovir is a synthetic nucleoside analogue active against herpesviruses
Adapalene	Inflammation	Retinoid Receptor	Adapalene is a third-generation topical retinoid primarily used in the treatment of acne.
Adefovir Dipivoxil (Preveon, Hepsera)	Infection	Reverse Transcriptase	Adefovir Dipivoxil (Preveon, Hepsera) works by blocking reverse transcriptase, an enzyme that is crucial for the hepatitis B virus (HBV) to reproduce in the body.
Adenine hydrochloride	Cancer	DNA/RNA Synthesis	Adenine hydrochloride is a hydrochloride salt form of adenine which is a purine derivative and a nucleobase with a variety of roles in biochemistry.
Adenosine (Adenocard)	Cardiovascular Disease	Others	Adenosine is a nucleoside composed of a molecule of adenine attached to a ribose sugar molecule (ribofuranose) moiety via a β-N9-glycosidic bond.
Adiphenine HCl	Cardiovascular Disease	Others	Adiphenine is a nicotinic receptor inhibitor with IC50 of 15 μM, used as an antispasmodic drug.
Adrenalone HCl	Cardiovascular Disease	Adrenergic Receptor	Adrenalone is an adrenergic agonist used as a topical vasoconstrictor and hemostatic, mainly acts on alpha-1 adrenergic receptors.
Adrucil (Fluorouracil)	Cancer	DNA/RNA Synthesis	Adrucil (Fluorouracil) belongs to the family of drugs called antimetabolites.
Afatinib (BIBW2992)	Cancer	EGFR,HER2	Afatinib (BIBW2992) irreversibly inhibits EGFR/HER2 including EGFR(wt), EGFR(L858R), EGFR(L858R/T790M) and HER2 with IC50 of 0.5 nM, 0.4 nM, 10 nM and 14 nM, respectively; 100-fold more active against Gefitinib-resistant L858R-T790M EGFR mutant. Phase 3.
Agomelatine		5-HT Receptor	Agomelatine is classified as a norepinephrine-dopamine disinhibitor (NDDI) due to its antagonism of the 5-HT2C receptor.
Albendazole (Albenza)	Vermifuge	Microtubule Associated	Albendazole (Albenza) is a member of the benzimidazole compounds used as a drug indicated for the treatment of a variety of worm infestations.
Albendazole Oxide (Ricobendazole)	Infection	Others	Albendazole Oxide is a tubulin polymerization or assembly inhibitor.

COMPOUND	INDICATION	TARGETS	BRIEF DESCRIPTION
Alfacalcidol	Endocrinology	Others	Alfacalcidol is a non-selective VDR activator medication.
Alfuzosin hydrochloride (Uroxatral)	Cardiovascular Disease	Adrenergic Receptor	Alfuzosin (Uroxatral) is an alpha1 receptor antagonist used to treat benign prostatic hyperplasia (BPH).
Alibendol	Neurological Disease	Others	Alibendol is an antispasmodic,chleretic, and cholekinetic.
Aliskiren hemifumarate	Cardiovascular Disease	RAAS	Aliskiren hemifumarate is a hemifumarate salt form of Aliskiren. Aliskiren is a novel orally effective direct renin inhibitor with an IC50 of 0.72 nM against renin.
Allopurinol (Zyloprim)	Neurological Disease	Others	Allopurinol (Zyloprim) is a clinically used xanthine oxidase (XO) inhibitor ,and its IC50 value is 0.78μM.
Allylthiourea	Metabolic Disease	Others	Allylthiourea is a metabolic inhibitor that selective inhibits ammonia oxidation.
Almotriptan malate (Axert)	Cardiovascular Disease	5-HT Receptor	Almotriptan malate (Axert) is a selective 5-hydroxytryptamine1B/1D (5-HT1B/1D) receptor agonist, used for the treatment of Migraine attacks in adults.
Alprostadil (Caverject)	Endocrinology	Others	Alprostadil(Caverject) is used as a drug in the treatment of erectile dysfunction and has vasodilatory properties.
Altrenogest	Neurological Disease	Estrogen/progestogen Receptor	Altrenogest is a progestogen structurally related to veterinary steroid trenbolone.
Altretamine (Hexalen)	Cancer	Others	Altretamine (Hexalen) is an anti-neoplastic agent.
Alverine Citrate	Digestive system disease	Others	Alverine citrate is a drug used for functional gastrointestinal disorders.
Amantadine hydrochloride (Symmetrel)	Cardiovascular Disease	Dopamine Receptor	Amantadine hydrochloride (Symmetrel) is used to treat or prevent infections of the respiratory tract caused by a certain virus
Ambrisentan	Neurological Disease	Endothelin Receptor	Ambrisentan, a highly selective antagonist of the endothelin-1 type A receptor with IC50 of 18 nM, is indicated for the treatment of pulmonary arterial hypertension (PAH).
Amfebutamone (Bupropion)	Infection	AChR,Dopamine Receptor	Amfebutamone (Bupropion) is a selective norepinephrine-dopamine reuptake inhibitor with IC50 of 6.5 and 3.4μM for the reuptake of dopamine and norepinephrine, respectively.
Amfenac Sodium (monohydrate)	Inflammation	COX	Amfenac Sodium monohydrate is a non-steroidal analgesic anti-inflammatory drug with acetic acid moiety.
AMG-073 HCl (Cinacalcet hydrochloride)	Endocrinology	CaSR	AMG-073 (Cinacalcet hydrochloride) represents a new class of compounds for the treatment of hyperparathyroidism.
Amidopyrine	Neurological Disease	Others	Amidopyrine is white crystalline substance used as an analgesic and antipyretic.

COMPOUND	INDICATION	TARGETS	BRIEF DESCRIPTION
Amiloride hydrochloride (Midamor)	Metabolic Disease	Sodium Channel	Amiloride hydrochloride (Midamor), a pyrazine compound inhibiting sodium reabsorption through sodium channels in renal epithelial cells.
Amiloride hydrochloride dihydrate	Cardiovascular Disease	Sodium Channel	Amiloride hydrochloride dihydrate is a potent epithelial sodium channel blocker.
Aminocaproic acid (Amicar)	Cardiovascular Disease	Others	Aminocaproic acid (Amicar) is a derivative and analogue of the amino acid lysine.
Aminoglutethimide (Cytadren)	Endocrinology	Aromatase	Aminoglutethimide (AMG, Cytadren) is an aromatase inhibitor with IC50 of 10 μM.
Aminophylline (Truphylline)	Respiratory Disease	PDE	Aminophylline (Phyllocontin, Truphylline) is a competitive nonselective phosphodiesterase inhibitor with an IC50 of 0.12 mM and also a nonselective adenosine receptor antagonist.
Aminosalicylate sodium	Neurological Disease	NF-κB	Sodium 4-Aminosalicylate is an antibiotic used to treat tuberculosis via NF-ÎºB inhibition and free radical scavenging.
Aminothiazole	Infection	Others	Aminothiazole can be used as a thyroid inhibitor and it has antibacterial activity.
Amiodarone HCl	Cardiovascular Disease	Potassium Channel,Autophagy	Amiodarone HCl is an antiarrhythmic drug for inhibition of ATP-sensitive potassium channel with IC50 of 19.1 μM.
Amisulpride	Neurological Disease	Dopamine Receptor	Amisulpride is an atypical antipsychotic used to treat psychosis in schizophrenia and episodes of mania in bipolar disorder.
Amitriptyline HCl	Infection	5-HT Receptor	Amitriptyline inhibits serotonin receptor, norepinephrine receptor, 5-HT4, 5-HT2 and sigma 1 receptor with IC50 of 3.45 nM, 13.3 nM, 7.31 nM, 235 nM and 287 nM, respectively.
Amlodipine (Norvasc)	Cardiovascular Disease	Calcium Channel	Amlodipine(Norvasc) is a long-acting calcium channel blocker with an IC50 of 1.9 nM.
Amlodipine besylate (Norvasc)	Cardiovascular Disease	Calcium Channel	Amlodipine (Norvasc) is a long-acting calcium channel blocker with an IC50 of 1.9 nM.
Ammonium Glycyrrhizinate		Dehydrogenase	Ammonium Glycyrrhizinate inhibits growth and cytopathology of several unrelated DNA and RNA viruses.
Amorolfine Hydrochloride	Infection	Others	Amorolfine hydrochloride is a antifungal reagent.
Amoxicillin (Amoxycillin)	Neurological Disease	Others	Amoxicillin is a moderate-spectrum, bacteriolytic, β-lactam antibiotic used to treat bacterial infections caused by susceptible microorganisms.
Amoxicillin sodium (Amox)	Infection	Others	Amoxicillin (Amox) is a moderate- spectrum, bacteriolytic, β-lactam antibiotic.
Amphotericin B		Others	Amphotericin B (AmB) is an amphipathic polyene antibiotic which permeabilizes ergosterol-containing membranes.

COMPOUND	INDICATION	TARGETS	BRIEF DESCRIPTION
Ampicillin sodium	Infection	Others	Ampicillin is a beta-lactam antibiotic that is part of the aminopenicillin family.
Ampicillin Trihydrate	Infection	Others	Ampicillin Trihydrate is a β-lactam antibiotic, which inhibits bacterial cell-wall synthesis (peptidoglycan cross-linking) by inactivating transpeptidases on the inner surface of the bacterial cell membrane.
Ampiroxicam	Cardiovascular Disease	COX	Ampiroxicam is a nonselective cyclooxygenase inhibitor uesd as anti-inflammatory drug.
Amprenavir (Agenerase)	Infection	HIV Protease	Amprenavir is an human immunodeficiency virus (HIV) protease inhibitor with IC50 of 14.6 ¡À 12.5 ng/ml for wild-type HIV isolate
Amprolium HCl	Metabolic Disease	Others	Amprolium chloride is a thiamin antagonist, which prevents carbohydrate synthesis by blocking thiamine uptake.
Anagrelide HCl	Endocrinology	PDE	Anagrelide is a drug used for the treatment of essential thrombocytosis.
Anastrozole	Endocrinology	Aromatase	Inhibits the enzyme aromatase
Aniracetam	Neurological Disease	AMPA Receptor-kainate Receptor-NMDA Receptor	Aniracetam is a nootropics and neuroprotective drug.
Anisotropine Methylbromide	Neurological Disease	Others	
Antazoline HCl	Neurological Disease	Others	Antazoline HCl is a first generation antihistamine, binding to the histamine H1 receptor and blocking the action of endogenous histamine.
Antipyrine	Infection	Others	Antipyrine is an analgesic and antipyretic agent.
Apatinib (YN968D1)	Cancer	VEGFR	Apatinib (YN968D1) is a small-molecule selective multitargeted tyrosine kinase inhibitor with an IC50 of 2.43 nM for the inhibition of VEGFR2.
Apixaban	Cardiovascular Disease	Factor Xa	Apixaban is a highly selective, reversible inhibitor of Factor Xa with Ki of 0.08 nM and 0.17 nM in human and rabbit, respectively.
Aprepitant (MK-0869)	Neurological Disease	Substance P	Substance P antagonists (SPA).
Arbidol HCl	Cardiovascular Disease	Others	Arbidol is an antiviral treatment for influenza infection.
Arecoline	Endocrinology	AChR	Arecoline is a muscarinic acetylcholine receptor agonist.
Argatroban	Cardiovascular Disease	Thrombin	Argatroban is an anticoagulant that is a small molecule direct thrombin inhibitor.

COMPOUND	INDICATION	TARGETS	BRIEF DESCRIPTION
Aripiprazole (Abilify)	Neurological Disease	5-HT Receptor	Aripiprazole is a human 5-HT1A receptor partial agonist with a Ki of 4.2 nM.
Artemether (SM-224)	Cancer	Others	Artemether is an antimalarial for the treatment of resistant strains of falciparum malaria.
Artemisinin	Infection	Others	Artemisinin is a drug used to treat multi-drug resistant strains of falciparum malaria.
Articaine HCl	Neurological Disease	Others	Articaine is a dental local anesthetic which contains an additional ester group that is metabolized by estearases in blood and tissue.
Asenapine	Neurological Disease	Adrenergic Receptor,5-HT Receptor	Asenapine (Saphris) is a new atypical antipsychotic used for the treatment of schizophrenia and acute mania associated with bipolar disorder .
Aspartame	Metabolic Disease	Others	Aspartame is an artificial, non-saccharide sweetener used as a sugar substitute in some foods and beverages.
Aspirin (Acetylsalicylic acid)	Cancer	COX	Aspirin is a salicylate drug, often used as an analgesic to relieve minor aches and pains, as an antipyretic to reduce fever, and as an anti-inflammatory medication.
Atazanavir sulfate	Cancer	HIV Protease	Atazanavir sulfate (BMS-232632-05) is a sulfate salt form of atazanavir (BMS-232632) that is an highly potent HIV protease inhibitor with EC50 and EC90 of 2.6~5.3 nM and 9~15 nM in cell culture.
Atomoxetine HCl	Neurological Disease	5-HT Receptor	Atomoxetine is a selective norepinephrine (NE) transporter inhibitor with Ki of 5 nM, with 15- and 290-fold lower affinity for human 5-HT and DA transporters.Phase 4.
Atorvastatin calcium (Lipitor)	Cardiovascular Disease	HMG-CoA Reductase	Atorvastatin (Lipitor) is an inhibitor of HMG-CoA reductase used as a cholesterol-lowering medication that blocks the production of cholesterol.
Atovaquone (Atavaquone)	Neurological Disease	Others	Atovaquone is a medication used to treat or prevent for pneumocystis pneumonia, toxoplasmosis, malaria, and babesia.
Atracurium besylate	Neurological Disease	Others	Atracurium besylate is a neuromuscular blocking agent with ED95 of 0.2 mg/kg.
Atropine	Respiratory Disease	AChR	Atropine sulfate monohydrate is a competitive muscarinic acetylcholine receptor antagonist with an IC50 of 2.5 nM.
Avanafil	Cardiovascular Disease	PDE	Avanafil is a highly selective PDE5 inhibitor with IC50 of 1 nM.
Avobenzone (Parsol 1789)		Others	Avobenzone(Parsol 1789) is an oil soluble ingredient used in sunscreen products to absorb the full spectrum of UVA rays and a dibenzoylmethane derivative.
Axitinib	Cancer	c-Kit,PDGFR,VEGFR	Axitinib blocked phosphorylation of VEGFR-2 and VEGFR-3 with average IC50s of 0.2 and 0.1 to 0.3 nM.
Azacitidine (Vidaza)	Cancer	DNA Methyltransferase	Azacitidine(Vidaza)and its deoxy derivative, decitabine (5-aza-2′deoxycytidine), are used in the treatment of myelodysplastic syndrome.

COMPOUND	INDICATION	TARGETS	BRIEF DESCRIPTION
Azacyclonol	Neurological Disease	Others	Azacyclonol, also known as γ-pipradol, is a drug used to diminish hallucinations in psychotic individuals.
Azaguanine-8	Cancer	Others	Azaguanine-8 is a purine analogs showing antineoplastic activity by competing with guanine in the metabolism.
Azaperone	Neurological Disease	Others	Azaperone is a pyridinylpiperazine and butyrophenone neuroleptic drug with sedative and antiemetic effects, which is used mainly as a tranquilizer in veterinary medicine.
Azatadine dimaleate	Infection	Histamine Receptor	Azatadine is an histamine and cholinergic inhibitor with IC50 of 6.5 nM and 10 nM, respectively.
Azathioprine (Azasan, Imuran)	Immunology	Rho	Azathioprine(Azasan, Imuran) is a drug that suppresses the immune system and is used in organ transplantation and autoimmune disease.
Azelastine hydrochloride (Astelin)	Neurological Disease	Histamine Receptor	Azelastine is a potent, second-generation, selective, histamine antagonist.
Azelnidipine	Neurological Disease	Calcium Channel	Azelnidipine is a dihydropyridine calcium channel blocker.
Azilsartan (TAK-536)	Neurological Disease	RAAS	Azilsartan (TAK-536) is an angiotensin II type 1 (AT1) receptor antagonist with IC50 of 2.6 nM.
Azilsartan Medoxomil (TAK-491)	Cardiovascular Disease	RAAS	Azilsartan Medoxomil is a potent angiotensin II type 1 (AT1) receptor antagonist, inhibits the RAAS, with an IC50 of 2.6 nM, exhibits >10,000-fold selectivity over AT2. Phase 3.
Azithromycin (Zithromax)	Cancer	Autophagy	Azithromycin (Zithromax) is an antibiotic for inhibition of parasite growth with IC50 of 8.4 μM.
Azithromycin Dihydrate	Infection	Others	Azithromycin Dihydrate is an acid stable orally administered macrolide antimicrobial drug, structurally related to erythromycin.
Azlocillin sodium salt	Neurological Disease	Others	Azlocillin is an acylampicillin with a broad spectrum against bacteria.
Aztreonam (Azactam, Cayston)	Infection	Others	Aztreonam (Azactam, Cayston) is a synthetic monocyclic beta-lactam antibiotic.
Bacitracin	Infection	Others	Bacitracin is a mixture of related cyclic polypeptides produced by organisms of the licheniformis group of Bacillus subtilis var Tracy, which disrupts both gram positive and gram negative bacteria by interfering with cell wall and peptidoglycan synthesis.
Balofloxacin	Metabolic Disease	Others	Balofloxacin is quinolone antibiotic, inhibiting the synthesis of bacterial DNA by interference with the enqyme DNA gyrase.
Bazedoxifene HCl	Metabolic Disease	Estrogen/progestogen Receptor	Bazedoxifene HCl is a novel, non-steroidal, indole-based estrogen receptor modulator (SERM) binding to both ERα and ERβ with IC50 of 23 nM and 89 nM.
Beclomethasone dipropionate	Inflammation	Glucocorticoid Receptor	Beclomethasone dipropionate is a potent glucocorticoid steroid used for the treatment of rhinitis and sinusitis.

COMPOUND	INDICATION	TARGETS	BRIEF DESCRIPTION
Bemegride		GABA Receptor	Bemegride is a central nervous system stimulant and antidote for barbiturate poisoning.
Benazepril hydrochloride	Cardiovascular Disease	RAAS	Benazepril is a medication used to treat high blood pressure.
Bendamustine HCL	Cancer	DNA/RNA Synthesis	Bendamustine inhibits SU-DHL-1 cell proliferation with IC50 at 50 μm.
Benidipine hydrochloride	Cardiovascular Disease	Calcium Channel	Benidipine HCl is a hydrochloride salt form of benidipine which is a dihydropyridine calcium channel blocker.
Benserazide	Neurological Disease	Others	Benserazide hydrochloride (Serazide) is a peripherally-acting aromatic L-amino acid decarboxylase (AAAD) or DOPA decarboxylase inhibitor.
Benzbromarone		P450 (e.g. CYP17)	Benzbromarone is a CYP2C9 inhibitor, it binds to CYP2C9 with Ki value of 19.3 nM.
Benzethonium chloride	Neurological Disease	AChR	Benzethonium chloride is a potent inhibitor of nAChRs, it inhibits α4β2 nAChRs and α7 nAChRs with IC50 of 49 nM and 122 nM, respectively.
Benzocaine	Respiratory Disease	Sodium Channel	Benzocaine is the ethyl ester of p-aminobenzoic acid (PABA), it is a local anesthetic commonly used as a topical pain reliever or in cough drops.
Benzoic acid		Others	Benzoic acid is a colorless crystalline solid and a simple aromatic carboxylic acid, used as a food preservative.
Benzthiazide	Cardiovascular Disease	Others	
Benztropine mesylate	Infection	Dopamine Receptor	Benztropine is a dopamine transporter (DAT) inhibitor with IC50 of 118 nM.
Benzydamine Hydrochloride	Inflammation	Others	Benzydamine hydrochloride is a topical nonsteroidal anti-inflammatory drug (NSAID) with anti-inflammatory, analgesic, antipyretic and local anesthetic activity.
Bephenium Hydroxynaphthoate	Vermifuge	Others	
Bepotastine Besilate	Cancer	Histamine Receptor	Bepotastine is a non-sedating, selective antagonist of histamine 1 (H1) receptor with pIC50 of 5.7.
Bergapten	Cancer	Others	Bergapten is a psoralen that can be photoactivated and is capable of crossing-linking DNA, covalently modifying proteins and lipids, and consequently inhibiting cell replication.
Beta Carotene		Others	Beta Carotene is an organic compound and classified as a terpenoid. It is a precursor (inactive form) of vitamin A.
Betahistine 2HCl		Histamine Receptor	Betahistine is a histamine H3 receptor inhibitor with IC50 of 1.9 μM.

COMPOUND	INDICATION	TARGETS	BRIEF DESCRIPTION
Betamethasone (Celestone)	Inflammation	Glucocorticoid Receptor	Betamethasone is a glucocorticoid steroid with anti-inflammatory and immunosuppressive properties.
Betamethasone Dipropionate		Glucocorticoid Receptor	Betamethasone Dipropionate is a glucocorticoid steroid with anti-inflammatory and immunosuppressive abilities.
Betamethasone valerate (Betnovate)	Inflammation	Glucocorticoid Receptor	Betamethasone Valerate is a moderately potent glucocorticoid steroid with anti-inflammatory and immunosuppressive properties.
Betamipron	Infection	Others	Betamipron is a chemical compound which is used together with panipenem to inhibit panipenem uptake into the renal tubule and prevent nephrotoxicity.
Betapar (Meprednisone)	Inflammation	Glucocorticoid Receptor	Betapar (Meprednisone) is a glucocorticoid and a methylated derivative of prednisone.
Betaxolol (Betoptic)	Neurological Disease	Adrenergic Receptor	Betaxolol is a selective beta1 adrenergic receptor blocker used in the treatment of hypertension and glaucoma.
Betaxolol hydrochloride (Betoptic)	Cardiovascular Disease	Adrenergic Receptor	Betaxolol is a β1 adrenergic receptor blocker with IC50 of 6 μM.
Bethanechol chloride	Neurological Disease	AChR	Carbamyl-beta-methylcholine chloride (Bethanechol chloride) is a selective muscarinic receptor agonist without any effect on nicotinic receptors.
Bexarotene	Cardiovascular Disease	Retinoid Receptor	Bexarotene is a retinoid specifically selective for retinoid X receptors, used as an oral antineoplastic agent in the treatment of cutaneous T-cell lymphoma.
Bextra (valdecoxib)	Neurological Disease	COX	Valdecoxib is a potent and selective inhibitor of COX-2 with IC50 of 5 nM.
Bezafibrate	Metabolic Disease	PPAR	Bezafibrate is the first clinically tested dual and pan-PPAR co-agonism.
BIBR-1048 (Dabigatran)	Infection	Thrombin	Dabigatran etexilate (BIBR-1048) is an anticoagulant from the class of the direct thrombin inhibitors.
Bicalutamide (Casodex)	Endocrinology	Androgen Receptor	An oral non-steroidal anti-androgen.
Bifonazole	Infection	Others	Bifonazole is an antifungal agent and a prostatic aromatase activity inhibitor with IC50 of 1.6 μM.
Bimatoprost	Cardiovascular Disease	Others	Bimatoprost is a prostaglandin analog used topically (as eye drops) to control the progression of glaucoma and in the management of ocular hypertension.
Bindarit	Cancer	Others	Bindarit exhibits selective inhibition against monocyte chemotactic proteins MCP-1/CCL2, MCP-3/CCL7 and MCP-2/CCL8.
Biotin (Vitamin B7)	Infection	Others	Biotin is a water-soluble B-vitamin and is necessary for cell growth, the production of fatty acids, and the metabolism of fats and amino acids.

COMPOUND	INDICATION	TARGETS	BRIEF DESCRIPTION
Bisacodyl	Cardiovascular Disease	Others	Bisacodyl (INN) is a stimulant laxative drug that works directly on the colon to produce a bowel movement.
Bisoprolol fumarate		Adrenergic Receptor	Bisoprolol fumarate is a selective type β1 adrenergic receptor blocker.
Bleomycin sulfate	Cancer	DNA/RNA Synthesis	A glycopeptide antibiotic produced by the bacterium Streptomyces verticillus.
Bortezomib (Velcade)	Cancer	Proteasome	Bortezomib also known as Velcade, MG-341, PS-341 is proteasome Inhibitor, effectively inhibits proteasome activity (Ki-0.6 nM).
Bosentan		Endothelin Receptor	Bosentan is an endothelin (ET) receptor antagonist for ET-A and ET-B with Ki of 4.7 nM and 95 nM, respectively.
Bosutinib (SKI-606)	Cancer	Src	Bosutinib (SKI-606) is a novel, dual Src/Abl inhibitor with IC50 of 1.2 nM and 1 nM, respectively.
Brinzolamide	Neurological Disease	Carbonic Anhydrase	Brinzolamide is a potent carbonic anhydrase II inhibitor with IC50 of 3.19 nM.
Bromhexine HCl	Cardiovascular Disease	Others	Bromhexine hydrochloride is a medication prescribed for coughs which works by dissolving hard phlegm.
Brompheniramine	Infection	Histamine Receptor	Brompheniramine is a histamine H1 receptors antagonist.
Broxyquinoline	Vermifuge	Others	Broxyquinoline is an antiprotozoal agent and able to release oxygen free radicals from the water in mucous membranes.
Brucine		Others	
Budesonide	Endocrinology	Glucocorticoid Receptor	Budesonide is a glucocorticoid steroid for the treatment of asthma, non-infectious rhinitis.
Bufexamac	Metabolic Disease	COX	Bufexamac is a COX inhibitor for IFN-α release with EC50 of 8.9 μM.
Buflomedil HCl	Neurological Disease	Others	Buflomedil is a vasodilator used to treat claudication or the symptoms of peripheral arterial disease.
Bumetanide	Cardiovascular Disease	Others	Bumetanide (Bumex) is a loop diuretic of the sulfamyl category to treat heart failure.
Bupivacaine hydrochloride (Marcain)	Neurological Disease	Sodium Channel	Bupivacaine hydrochloride (Marcain) is a more potent cAMP production inhibitor with an IC50 of 2.3 μM.
Busulfan (Myleran, Busulfex)	Cardiovascular Disease	Others	Busulfan (Myleran, Busulfex) is a cell cycle non-specific alkylating antineoplastic agent.

COMPOUND	INDICATION	TARGETS	BRIEF DESCRIPTION
Butenafine HCl	Neurological Disease	Others	Butenafine HCl is a synthetic benzylamine antifungal, works by inhibiting the synthesis of sterols by inhibiting squalene epoxidase.
Butoconazole nitrate	Infection	Others	Butoconazole nitrate is an anti-fungal agent for IL-2, TNFα, IFN and GM-CSF with IC50 of 7.2 μg/mL, 14.4 μg/mL, 7.36 μg/mL and 7.6 μg/mL, respectively.
Cabazitaxel (Jevtana)	Neurological Disease	Microtubule Associated	Cabazitaxel (Jevtana, XRP6258) is a semi-synthetic derivative of a natural taxoid.
Calcitriol (Rocaltrol)	Endocrinology	Others	Calcitriol is the hormonally active form of vitamin D.
Calcium Gluceptate		Others	
Camptothecin	Cancer	Topoisomerase	Camptothecin (CPT) is a cytotoxic quinoline alkaloid which inhibits the DNA enzyme topoisomerase I (topo I) with an IC50 and IC70 of 50 nM and 0. 225 μM .
Camylofin Chlorhydrate	Digestive system disease	Others	
Candesartan (Atacand)	Cardiovascular Disease	RAAS	Candesartan (Atacand) is an angiotensin II receptor antagonist with an IC50 of 15 μg/kg
Candesartan cilexetil (Atacand)	Cardiovascular Disease	RAAS	Candesartan Cilexetil is a specific nonpeptide Ang II receptor (ATR) antagonist and the prodrug of candesartan which is an ATR antagonist with an IC50 of 15 μg/kg
Capecitabine (Xeloda)	Cancer	DNA/RNA Synthesis	Capecitabine (Xeloda) is an orally-administered chemotherapeutic agent (IC50 = 0.7~5 mM) and prodrug of 5-fluorouracil which is a DNA synthesis inhibitor with an IC50 for 5.0 ± 1.8 μM.
Captopril (Capoten)	Metabolic Disease	RAAS	Captopril (Capoten) is an angiotensin-converting enzyme (ACE) inhibitor with IC50 of 6 nM.
Carbachol		Others	
Carbadox	Infection	Others	
Carbamazepine (Carbatrol)	Neurological Disease	Sodium Channel,Autophagy	Carbamazepine is a sodium channel blocker with an IC50 of 140 μM.
Carbazochrome sodium sulfonate	Cancer	Others	Carbazochrome is an antihemorrhagic for use in the treatment of hemorrhoids.
Carbenicillin disodium	Infection	Others	Carbenicillin is a semi-synthetic penicillin antibiotic which interferes with cell wall synthesis of gram-negative bacteria while displaying low toxicity.
Carbenoxolone Sodium	Endocrinology	Others	

COMPOUND	INDICATION	TARGETS	BRIEF DESCRIPTION
Carbidopa	Neurological Disease	Others	Carbidopa is an aromatic-L-amino-acid decarboxylase inhibitor with an IC50 of 29 ± 2 μM.
Carbimazole	Infection	Others	Carbimazole is an imidazole antithyroid agent.
Carfilzomib (PR-171)	Cardiovascular Disease	Proteasome	Carfilzomib (PR-171) is an irreversible proteasome inhibitor with IC50 of <5 nM, displayed preferential in vitro inhibitory potency against the ChT-L activity in the β5 subunit, but little or no effect on the PGPH and T-L activities.
Carmofur	Cancer	DNA/RNA Synthesis	Carmofur (INN) is a pyrimidine analogue used as an antineoplastic agent.
Carprofen	Inflammation	COX	Carprofen inhibits canine COX2 with IC50 of 0.03 mM.
Carvedilol	Cardiovascular Disease	Adrenergic Receptor	Carvedilol is a non-selective beta blocker/alpha-1 blocker with an IC50 of 3.8 μM for inhibition of LDL oxidation.
Caspofungin acetate	Infection	Others	Caspofungin acetate is a lipopeptide antifungal drug.
Catharanthine		Achr	Catharanthine inhibits nicotinic receptor mediated diaphragm contractions with IC50 of 59.6 μM.
Cefdinir (Omnicef)	Infection	Others	Cefdinir (Omnicef) is a bacteriocidal antibiotic.
Cefditoren pivoxil	Infection	Others	Cefditoren pivoxil is used to treat uncomplicated skin and skin structure infections, etc.
Cefoperazone (Cefobid)	Infection	Others	Cefoperazone is a cephalosporin antibiotic for inhibition of rMrp2-mediated [3H]E217βG uptake with IC50 of 199 μM.
Ceftazidime Pentahydrate	Infection	Others	
Ceftiofur HCl		Others	Ceftiofur HCl is a cephalosporin antibiotic, used to treat both Gram-positive and Gram-negative bacteria infection.
Celecoxib	Inflammation	COX	COX-2 inhibitor,IC50=0.04uM
Cephalexin (Cefalexin)	Infection	Others	Cefalexin is a cephalosporin antibiotic.
Cephalomannine	Cancer	Others	Cephalomannine is an active anti-cancer agent obtained from Taxus yunnanensis and has an antineoplastic effect on tumors found in mice.
Cepharanthine	Metabolic Disease	Others	Cepharanthine is a biscoclaurine alkaloid inhibiting tumor necrosis factor (TNF)-α-mediated NFκB stimulation, plasma membrane lipid peroxidation and platelet aggregation and suppressing cytokine production.

COMPOUND	INDICATION	TARGETS	BRIEF DESCRIPTION
Cetirizine Dihydrochloride	Inflammation	Histamine Receptor	Cetirizine is an antihistamine.
Cetrimonium Bromide	Infection	Others	Cetrimonium Bromide is a known component of the broad-spectrum antiseptic cetrimide, which is a mixture of different quaternary ammonium salts.
Cetylpyridinium Chloride	Infection	Others	Cetylpyridinium chloride is a cationic quaternary ammonium compound used as oropharyngeal antiseptic.
Chenodeoxycholic acid	Infection	Others	Chenodeoxycholic acid is a naturally occurring human bile acid.
Chloramphenicol (Chloromycetin)	Infection	Others	Chloramphenicol (Chloromycetin) is a bacteriostatic antimicrobial.
Chlormezanone (Trancopal)	Respiratory Disease	Others	Chlormezanone(Trancopal), a non-benzodiazepine that is used in the management of anxiety. It has been suggested for use in the treatment of muscle spasm.
Chlorocresol		Others	Chlorocresol is an activator of ryanodine receptor
Chlorothiazide	Cardiovascular Disease	Others	Chlorothiazide is a diuretic and antihypertensive. (IC50=3.8 mM)
Chloroxine	Infection	Others	Chloroxine is a synthetic antibacterial compound that is effective in the treatment of dandruff and seborrheic dermatitis when incorporated in a shampoo.
Chlorpheniramine Maleate	Neurological Disease	Histamine Receptor	Chlorpheniramine (Chlorpheniramine maleate, Chlorphenamine) is an histamine H1 receptor antagonist with IC50 of 12 nM.
Chlorpromazine (Sonazine)	Neurological Disease	Dopamine Receptor,Potassium Channel	Chlorpromazine hydrochloride (Sonazine) is a dopamine and potassium channel inhibitor with IC50 of 6.1 and 16 μM for nward-rectifying K+ currents and time-independent outward currents.
Chlorpropamide	Infection	Others	Chlorpropamide is a sulfonylurea class drug for type 2 diabetes mellitus.
Chlorprothixene	Neurological Disease	Dopamine Receptor	Chlorprothixene is a typical antipsychotic drug of the thioxanthene class and was the first of the series to be synthesized.
Chlorquinaldol	Infection	Others	Chlorquinaldol is an antimicrobial agent used for local antisepsy.
Chlorzoxazone	Metabolic Disease	Others	Chlorzoxazone is a muscle-relaxing drug,and a probe for human liver cytochrome P-450IIE1.
Choline Chloride		Others	Choline chloride is a quaternary ammonium salt used as an additive for animal feed.
Chromocarb	Cardiovascular Disease	Others	Chromocarb is a vasoprotectant.

COMPOUND	INDICATION	TARGETS	BRIEF DESCRIPTION
Ciclopirox (Penlac)	Neurological Disease	ATPase	Ciclopirox (Penlac) is a synthetic antifungal agent.
Ciclopirox ethanolamine	Infection	ATPase	Ciclopirox ethanolamine (Ciclopirox olamine, HOE 296) is a broad-spectrum antifungal agent working as an iron chelator.
Cilnidipine	Cardiovascular Disease	Calcium Channel	Cilnidipine is a calcium channel blocker.
Cilostazol	Cardiovascular Disease	PDE	Cilostazol is a medication used in the alleviation of the symptom of intermittent claudication in individuals with peripheral vascular disease.
Cimetidine (Tagamet)	Inflammation	Histamine Receptor	Cimetidine (Tagamet), a histamine congener, competitively inhibits histamine binding to histamine H2 receptors.
Cinchophen	Immunology	Others	Cinchophen is an analgesic drug that is frequently used to treat gout.
Cinepazide maleate	Inflammation	Calcium Channel	Cinepazide maleate is a maleate salt form of cinepazide which is a vasodilator.
Cisatracurium besylate (Nimbex)	Neurological Disease	Adrenergic Receptor	Cisatracurium besylate is a nondepolarizing neuromuscular blocking agent, antagonizing the action of acetylcholine by inhibiting neuromuscular transmission.
Cisplatin		DNA/RNA Synthesis	Cisplatin is an inorganic platinum complex, which is able to inhibit DNA synthesis by conforming DNA adducts in tumor cells.
Cladribine	Cancer	DNA/RNA Synthesis	Cladribine (Leustatin) is an adenosine deaminase inhibitor with an IC50 of about 0.2 μM.
Clarithromycin (Biaxin, Klacid)	Neurological Disease	P450 (e.g. CYP17)	Clarithromycin is a macrolide antibiotic and a CYP3A4 substrate and inhibitor.
Clemastine Fumarate	Immunology	Histamine Receptor	Clemastine fumarate, a histamine H1 antagonist used as the hydrogen fumarate in hay fever, rhinitis, allergic skin conditions, and pruritus.
Cleviprex (Clevidipine)	Cardiovascular Disease	Calcium Channel	Cleviprex (Clevidipine) is a dihydropyridine calcium channel blocker use as agent for the reduction of blood pressure.
Climbazole	Infection	Others	Climbazole is a broad-spectrum imidazole antifungal agent that can provide anti-dandruff benefits.
Clindamycin	Infection	Others	Clindamycin inhibits protein synthesis by acting on the 50S ribosomal.
Clindamycin hydrochloride (Dalacin)	Neurological Disease	Others	Clindamycin hydrochloride (Dalacin) is the hydrated hydrochloride salt of clindamycin which is a semisynthetic antibiotic.
Clindamycin palmitate HCl	Infection	Others	Clindamycin palmitate HCl is a water soluble hydrochloride salt of the ester of clindamycin and palmitic acid and a lincosamide antibiotic.

COMPOUND	INDICATION	TARGETS	BRIEF DESCRIPTION
Clobetasol propionate	Neurological Disease	Glucocorticoid Receptor	Clobetasol propionate is a anti-inflammatory corticosteroid used to treat various skin disorders.
Clofarabine	Cancer	DNA/RNA Synthesis	Inhibits the enzymatic activities of ribonucleotide reductase (IC50 = 65 nM) and DNA polymerase
Clofazimine	Infection	Phospholipase (e.g. PLA)	Clofazimine is a rhimophenazine dye, originally developed for the treatment of tuberculosis, it has both antimicrobial and antiinflammatory activity, postulated mechanisms of action include intercalation of clofazimine with bacterial DNA and increasing levels of cellular phospholipase A2.
Clofibric acid	Metabolic Disease	PPAR	Clofibric acid is a PPARα agonist and hypolipidemic agent.
Clofoctol	Infection	Others	
Clomifene citrate (Serophene)	Cancer	Estrogen/progestogen Receptor	Clomifene citrate (Serophene) is a selective estrogen receptor modulator.
Clomipramine HCl		5-HT Receptor	Clomipramine HCl is a hydrochloride salt of clomipramine which is a serotonin transporter (SERT), norepinephrine transporter (NET) dopamine transporter (DAT) blocker with Ki of 0.14, 54 and 3 nM, respectively.
Clonidine hydrochloride (Catapres)	Infection	Autophagy,Adrenergic Receptor	Clonidine hydrochloride (Catapres) is a direct-acting α2 adrenergic agonist with an ED50 of 0.02±0.01 mg/kg.
Clopidogrel (Plavix)	Cardiovascular Disease	P2 Receptor	Clopidogrel is an oral, thienopyridine class antiplatelet agent.
Clorprenaline HCL	Cardiovascular Disease	Adrenergic Receptor	Clorprenaline HCl is a β2-receptor agonist, it has a significant expansion of the bronchial effect.
Clorsulon	Cancer	Others	Clorsulon is a competitive 8-phosphoglycerate kinase and phospho-glyceromutase inhibitor.
Closantel	Vermifuge	Others	Closantel is gram positive antibacterial activity inhibitor, inhibiting the KinA/Spo0F system with IC50 of 3.8 μM.
Closantel Sodium	Vermifuge	Others	Closantel is gram positive antibacterial activity inhibitor, inhibiting the KinA/Spo0F system with IC50 of 3.8 μM.
Clotrimazole (Canesten)	Infection	Others	Clotrimazole (Canesten) is a synthetic, antifungal and broad-spectrum derivate of imidazole.
Cloxacillin sodium (Cloxacap)	Cardiovascular Disease	Others	Cloxacillin sodium (Cloxacap) is a sodium salt of cloxacillin (Cloxapen, Cloxacap□Orbenin) that is a penicillinase-resistant, acid resistant, semi-synthetic penicillin.
Clozapine (Clozaril)	Cardiovascular Disease	5-HT Receptor	Clozapine (Clozaril) is a potent 5-HT1C receptor antagonist with an IC50 of 110 nM for 5-HT-stimulated phosphoinositide hydrolysis.
Cobicistat (GS-9350)	Cancer	P450 (e.g. CYP17)	Cobicistat (GS-9350) is a potent and selective inhibitor of CYP3A with IC50 of 30-285 nM.

COMPOUND	INDICATION	TARGETS	BRIEF DESCRIPTION
Conivaptan HCl (Vaprisol)	Cardiovascular Disease	Vasopressin Receptor	Conivaptan is a non-peptide inhibitor of antidiuretic hormone (vasopressin receptor antagonist).
Cortisone acetate (Cortone)	Cancer	Glucocorticoid Receptor	Cortisone acetate (Cortone) is an acetate salt form of cortisone that is a steroid hormone and a glucocorticoid.
Coumarin		Others	Coumarin is a secondary phytochemical with hepatotoxic and carcinogenic properties.
Crizotinib (PF-02341066)	Cancer	ALK,c-Met	PF-2341066 (Crizotinib) is a potent inhibitor of c-Met and ALK with IC50 of 11 nM and 24 nnM, respectivley.
Crystal violet	Infection	Others	Crystal violet is a triarylmethane dye.
Curcumin		Others	Curcumin is the principal curcuminoid of the popular Indian spice turmeric, which is a member of the ginger family (Zingiberaceae).
Cyclamic acid	Inflammation	Others	Cyclamic acid in the form of its sodium or calcium salt is one of the most widely used artificial sweeteners.
Cyclandelate	Neurological Disease	Others	Cyclandelate is an effective inhibitor of rat hepatic acycloenzyme A: cholesterol acyltransferase (ACAT) with IC50 of 80 μM.
Cyclophosphamide monohydrate	Cancer	Others	Cyclophosphamide is a nitrogen mustard alkylating agent, it attaches the alkyl group to the guanine base of DNA.
Cyclosporine (Neoral)	Immunology	Others	Cyclosporine (Neoral) is an immunosuppressant drug.
Cyproheptadine HCl (Periactin)	Neurological Disease	Histamine Receptor	Cyproheptadine hydrochloride (Periactin) is a hydrochloride salt form of cyproheptadine which is a histamine receptor antagonist for 5-HT2 receptor with IC50 of 0.6 nM.
Cyromazine	Vermifuge	Others	Cyromazine is a triazine insect growth regulator used as an insecticide and an acaricide.
Cysteamine HCl	Metabolic Disease	Others	Cysteamine is an agent for the treatment of nephropathic cystinosis and an antioxidant.
Cytidine	Cardiovascular Disease	Others	Cytidine is a nucleoside molecule that is formed when cytosine is attached to a ribose ring, cytidine is a component of RNA.
D-Mannitol (Osmitrol)	Cardiovascular Disease	Others	D-Mannitol(Osmitrol) is an osmotic diuretic agent and a weak renal vasodilator.
Dabrafenib (GSK2118436)	Infection	Raf	Dabrafenib (GSK2118436) is a mutant BRAFV600 specific inhibitor with IC50 of 0.8 nM, with 4- and 6-fold less potency against B-Raf(wt) and c-Raf, respectively. Phase 3.
Dacarbazine (DTIC-Dome)	Cancer	DNA/RNA Synthesis	Dacarbazine (DTIC-Dome) is an antineoplastic chemotherapy drug used in the treatment of various cancers.

COMPOUND	INDICATION	TARGETS	BRIEF DESCRIPTION
Daidzein	Cardiovascular Disease	Others	Daidzein belongs to the group of isoflavones.
Dapoxetine hydrochloride (Priligy)	Neurological Disease	5-HT Receptor	Dapoxetine hydrochloride is a short-acting novel selective serotonin reuptake inhibitor.
DAPT (GSI-IX)	Cancer	Gamma-secretase,Beta Amyloid	DAPT (GSI-IX) is a novel γ-secretase inhibitor, which inhibits Aβ production with IC50 of 20 nM in HEK 293 cells.
Darifenacin HBr	Infection	AChR	Darifenacin is a selective M3 muscarinic receptor antagonist with pKi of 8.9.
Darunavir Ethanolate (Prezista)	Infection	HIV Protease	Darunavir Ethanolate (Prezista) is an HIV protease inhibitor.
Dasatinib (BMS-354825)	Cancer	Bcr-Abl,Src,c-Kit	Dasatinib also known as BMS-354825, Sprycel, BMS354825 is ATP-competitive, dual SRC/ABL inhibitor. BMS-354825 inhibits all members of the Src family, including c-Src, Lck, Fyn, and Yes (IC50 < 1.1nmol/L).
Daunorubicin HCl (Daunomycin HCl)	Cancer	Topoisomerase	Daunorubicin HCl inhibits both DNA and RNA synthesis and inhibits DNA synthesis with Ki of 0.02 μM.
Decamethonium bromide	Neurological Disease	AChR	Decamethonium Bromide is a nicotinic AChR partial agonist and neuromuscular blocking agent.
Decitabine	Cardiovascular Disease	DNA Methyltransferase	Decitabine (NSC 127716, Dacogen, DAC) is an available nucleoside-based DNA methyltransferase (DNMT) inhibitor with IC50 of 490, 400 and 100 nM for A549, LoVo and LoVo-DX cell lines.
Deferasirox (Exjade)	Endocrinology	Others	Deferasirox(Exjade) is a rationally-designed oral iron chelator.
Deflazacort (Calcort)	Endocrinology	Glucocorticoid Receptor	Deflazacort (Calcort) is a glucocorticoid used as an anti-inflammatory and immunosuppressant.
Dehydroepiandrosterone (DHEA)	Endocrinology	Estrogen/progestogen Receptor,Androgen Receptor	Dehydroepiandrosterone(DHEA) is a 19-carbon endogenous natural steroid hormone.
Deoxyarbutin	Cardiovascular Disease	Others	DeoxyArbutin is a reversible tyrosinase inhibitor, inhibiting tyrosinase activity with IC50 of 50 nM.
Deoxycorticosterone acetate	Endocrinology	Others	Deoxycorticosterone acetate is a steroid hormone used for intramuscular injection for replacement therapy of the adrenocortical steroid.
Desloratadine	Cardiovascular Disease	Histamine Receptor	Desloratadine is a potent antagonist for human histamine H1 receptor with IC50 of 51 nM.
Desonide	Inflammation	Glucocorticoid Receptor	Desonide is a low potency topical corticosteroid.
Detomidine HCl	Cardiovascular Disease	Adrenergic Receptor	Detomidine produce dose-dependent sedative and analgesic effects, mediatated by activation of α2 catecholamine receptors.

COMPOUND	INDICATION	TARGETS	BRIEF DESCRIPTION
Dexamethasone	Inflammation	IL Receptor,Autophagy	Dexamethasone is an anti-inflammatory and immunosuppressant.
Dexamethasone acetate	Inflammation	Autophagy,IL Receptor	Dexamethasone is a potent synthetic member of the glucocorticoid class of steroid drugs used as an anti-inflammatory and immunosuppressant.
Dexlansoprazole	Cardiovascular Disease	Proton Pump	Dexlansoprazole, the dextrorotatory enantiomer of lansoprazole, is a proton pump inhibitor (PPI) formulated to have dual delayed-release properties.
Dexmedetomidine	Neurological Disease	Adrenergic Receptor	Dexmedetomidine is a sedative medication used by intensive care units and anesthetists.
Dexmedetomidine HCl (Precedex)	Neurological Disease	Adrenergic Receptor	Dexmedetomidine is a highly selective and potent alpha-2 adrenoceptor agonist, which reduces anesthetic requirements for patients by providing significant sedation.
Dexrazoxane Hydrochloride	Cardiovascular Disease	Others	A cardioprotective agent
Dextrose (D-glucose)	Infection	Others	Dextrose (D-glucose), a simple sugar (monosaccharide), is an important carbohydrate in biology.
Dibenzothiophene		Others	Dibenzothiophene (DBT) is a model compound for organic sulfur in fossil fuels.
Dibucaine HCL	Endocrinology	Sodium Channel	Dibucaine (Cinchocaine) HCl is a local anesthetics.
Diclazuril	Infection	Others	Diclazuril is an anti-coccidial drug.
Diclofenac	Neurological Disease	COX	Diclofenac is a non-selective COX inhibitor with IC50 of 60 and 220 nM for ovine COX-1 and -2, respectively.
Diclofenac Diethylamine	Neurological Disease	Others	Diclofenac diethylamine is a nonsteroidal anti-inflammatory drug taken to reduce inflammation and as an analgesic reducing pain in certain conditions.
Diclofenac Potassium	Infection	Others	Diclofenac potassium is a nonsteroidal anti-inflammatory drug taken to reduce inflammation and as an analgesic reducing pain in certain conditions.
Dicloxacillin Sodium	Infection	Others	Dicloxacillin is a β-lactamase resistant penicillin similar to oxacillin and it has activity against gram-positive/negative aerobic and anaerobic bacteria.
Dicyclomine HCl	Neurological Disease	Others	
Didanosine (Videx)	Infection	Reverse Transcriptase	Didanosine (Videx, Videx EC) is a reverse transcriptase inhibitor with an IC50 of 0.49 μM.
Dienogest	Endocrinology	Estrogen/progestogen Receptor	Dienogest is an orally active synthetic progesterone (or progestin).

COMPOUND	INDICATION	TARGETS	BRIEF DESCRIPTION
Diethylstilbestrol (Stilbestrol)	Cancer	Estrogen/progestogen Receptor	Diethylstilbestrol(Stilbestrol), a synthetic nonsteroidal estrogen used in the treatment of menopausal and postmenopausal disorders.
Difloxacin HCl	Infection	Others	
Difluprednate	Endocrinology	Others	Difluprednate (difluoroprednisolone butyrate acetate, or DFBA) is a synthetic difluorinated prednisolone derivative, it originally developed for dermatologic applications.
Diltiazem HCl (Tiazac)	Cardiovascular Disease	Calcium Channel	Diltiazem HCl (Tiazac) is a benzothiazepine derivative with vasodilating action due to its antagonism of the actions of the calcium ion in membrane functions.
Dimethyl Fumarate	Inflammation	Others	Dimethyl fumarate is a promising novel oral therapeutic option shown to reduce disease activity and progression in patients with relapsing-remitting multiple sclerosis.
Diminazene Aceturate	Vermifuge	Others	Diminazene is a di-amidine also known as 4,4-(1-Triazene–1,3–diyl)bis(benzenecarboximidamide), used as an effective trypanocidal agent.
Diperodon HCl	Neurological Disease	Others	
Diphemanil methylsulfate	Neurological Disease	AChR	Diphemanil Methylsulfate is a quaternary ammonium anticholinergic, it binds muscarinic acetycholine receptors (mAchR).
Diphenhydramine HCl (Benadryl)	Immunology	Histamine Receptor	Diphenhydramine hydrochloride (Benadryl), a histamine H1 antagonist used as an antiemetic, antitussive, for dermatoses and pruritus, for hypersensitivity reactions, as a hypnotic, an antiparkinson, and as an ingredient in common cold preparations.
Diphenylpyraline HCl	Neurological Disease	Others	
Dipyridamole (Persantine)	Cardiovascular Disease	PDE	Dipyridamole (Permole, Persantine) is a phosphodiesterase inhibitor that blocks uptake and metabolism of adenosine by erythrocytes and vascular endothelial cells.
Dirithromycin	Infection	Others	Dirithromycin is a macrolide glycopeptide antibiotic by binding to the 50S subunit of the 70S bacterial ribosome to inhibit the translocation of peptides.
Disopyramide Phosphate	Cardiovascular Disease	Others	
Disulfiram (Antabuse)	Neurological Disease	Dehydrogenase	Disulfiram (Antabuse) is a drug used to support the treatment of chronic alcoholism by producing an acute sensitivity to alcohol.
Divalproex sodium	Neurological Disease	Autophagy	Divalproex sodium consists of a compound of sodium valproate and valproic acid in a 1:1 molar relationship in an enteric coated form.
DL-Carnitine hydrochloride	Cardiovascular Disease	Others	DL-Carnitine hydrochloride is a quaternary ammonium compound biosynthesized from the amino acids lysine and methionine.
Docetaxel (Taxotere)	Cancer	Microtubule Associated	An microtubule disassembly inhibitor with IC50 of a range of 0.31–100 ηM.

COMPOUND	INDICATION	TARGETS	BRIEF DESCRIPTION
Dofetilide (Tikosyn)	Cardiovascular Disease	Potassium Channel	Dofetilide(Tikosyn) is a class III antiarrhythmic agent.
Domiphen Bromide	Infection	Others	Domiphen bromide is a quaternary ammonium antiseptic with actions as a cationic surfactant.
Domperidone (Motilium)	Neurological Disease	Dopamine Receptor	Domperidone (Motilium) is a dopamine blocker and an antidopaminergic reagent.
Dopamine hydrochloride (Inotropin)	Infection	Dopamine Receptor	Dopamine hydrochloride (Inotropin) is a catecholamine neurotransmitter present in a wide variety of animals,And a dopamine D1-5 receptors agonist.
Doripenem Hydrate	Infection	Others	Doripenem (doripenem monohydrate) is an ultra-broad spectrum injectable antibiotic.
Doxapram HCl	Neurological Disease	Others	Doxapram HCl inhibits TASK-1, TASK-3, TASK-1/TASK-3 heterodimeric channel function with EC50 of 410 nM, 37 μM, 9 μM, respectively.
Doxazosin mesylate	Cardiovascular Disease	Adrenergic Receptor	Doxazosin mesylate (Cardura) is an alpha-1 adrenergic receptor blocker.
Doxercalciferol (Hectorol)	Endocrinology	Others	Doxercalciferol (Hectorol) is a synthetic analog of vitamin D.
Doxifluridine	Immunology	Others	Doxifluridine is a thymidine phosphorylase activator for PC9-DPE2 cells with IC50 of 0.62 μM.
Doxofylline	Metabolic Disease	PDE	Doxofylline is a phosphodiesterase inhibitor and a xanthine derivative drug for asthma.
Doxorubicin (Adriamycin)	Cancer	Autophagy,Topoisomerase	Doxorubicin is a topoisomerase II inhibitor with IC50 of 1 and 2 μM for the inhibition of MCF-7 and MDA-MB231.
Doxylamine Succinate	Neurological Disease	Histamine Receptor	Doxylamine succinate competitively inhibits histamine at H1 receptors with substantial sedative and anticholinergic effects.
Dronedarone HCl (Multaq)	Neurological Disease	Potassium Channel,Sodium Channel,Calcium Channel	Dronedarone is a therapy for the treatment of patients with paroxysmal and persistent atrial fibrillation or atrial flutter.
Droperidol	Neurological Disease	Others	Droperidol is a potent antagonist of dopamine subtype 2 receptors in the limbic system.
Dropropizine	Respiratory Disease	Others	Dropropizine is a racemic non-opiate antitussive agent, it is used as a cough suppressant.
Drospirenone	Endocrinology	Estrogen/progestogen Receptor	Drospirenone is a synthetic progestin that is an analog to spironolactone.
Duloxetine HCl (Cymbalta)	Neurological Disease	5-HT Receptor	Duloxetine is a serotonin-norepinephrine reuptake inhibitor with Ki of 4.6 nM, used for treatment of major depressive disorder and generalized anxiety disorder (GAD).

COMPOUND	INDICATION	TARGETS	BRIEF DESCRIPTION
Dutasteride	Endocrinology	5-alpha Reductase	5-alpha-reductase inhibitor
Dyclonine HCl	Inflammation	Others	Dyclonine hydrochloride is a hydrochloride salt form of dyclonine which is an oral anaesthetic.
Dydrogesterone	Endocrinology	Estrogen/progestogen Receptor	Dydrogesterone is an orally active progestogen which acts directly on the uterus, producing a complete secretory endometrium in an estrogen-primed uterus.
Dyphylline (Dilor)	Respiratory Disease	PDE	Dyphylline (Dilor, Lufyllin, diprophylline) is a xanthine derivative with bronchodilator and vasodilator effects.
Econazole nitrate (Spectazole)	Neurological Disease	Calcium Channel	Econazole nitrate (Spectazole) is an imidazole class antifungal medication.
Edaravone (MCI-186)	Cardiovascular Disease	Others	Edaravone(MCI-186), a strong novel free radical scavenger, is used for treatment of patients with acute brain infarction.
Eltrombopag (SB-497115-GR)	Cancer	Others	Eltrombopag (SB-497115-GR, Promacta, Revolade) is a small molecule agonist of the c-mpl (TpoR) receptor with an IC50 of 0.69 μM for the inhibition of hERG K+ channel tail current.
Elvitegravir (GS-9137)	Immunology	Integrase	Elvitegravir is a human immunodeficiency virus integrase inhibitor with EC50 of 0.7, 2.8 and 1.4 for HIV-1 IIIB, HIV-2 EHO and HIV-2 ROD.
Emtricitabine (Emtriva)	Infection	Reverse Transcriptase	Emtricitabine (Emtriva) is a nucleoside reverse transcriptase inhibitor with an IC50 of 27.7 μM.
Enalapril maleate (Vasotec)	Cardiovascular Disease	RAAS	Enalapril maleate (Vasotec), the active metabolite of enalapril, competes with angiotensin I for binding at the angiotensin-converting enzyme, blocking the conversion of angiotensin I to angiotensin II.
Enalaprilat dihydrate	Cardiovascular Disease	RAAS	Enalaprilat is the first dicarboxylate-containing ACE inhibitor with an IC50 of 4 nM.
Enoxacin (Penetrex)	Infection	Topoisomerase	Enoxacin(Penetrex)is an oral broad-spectrum fluoroquinolone antibacterial agent used in the treatment of urinary tract infections and gonorrhea. Insomnia is a common adverse effect.
Entacapone	Neurological Disease	Histone Methyltransferase	Entacapone inhibits catechol-O-methyltransferase(COMT) with IC50 of 151 nM.
Entecavir hydrate	Infection	Reverse Transcriptase	Entecavir hydrate belongs to the family of medicines called antivirals.
Epalrestat	Inflammation	Others	Epalrestat is an aldose reductase inhibitor with IC50 of 72 nM.
Epinephrine bitartrate (Adrenalinium)	Cancer	Adrenergic Receptor	Epinephrine bitartrate (D02149, Adrenalinium) is alpha- and beta-adrenergic receptor stimulator.
Epirubicin HCl		Topoisomerase	Epirubicin HCl, a semisynthetic L-arabino derivative of doxorubicin, is an antineoplastic agent by inhibiting Topoisomerase.

COMPOUND	INDICATION	TARGETS	BRIEF DESCRIPTION
Eprosartan Mesylate	Cardiovascular Disease	RAAS	Eprosartan is a nonpeptide angiotensin II receptor antagonist, [3H]-eprosartan binds to the AT1 receptor with KD of 0.83 nM in rat vascular smooth muscle cells.
Erdosteine	Respiratory Disease	Others	Erdosteine is a mucolytic which is used in treatment of excessive viscous mucus.
Erlotinib HCl	Cancer	EGFR,Autophagy	Erlotinib also known as Tarceva, CP-358774, OSI-774, NSC-718781 is a HCL salt with IC50 of 2 nM for HER1/EGFR TK.
Erythromycin (E-Mycin)	Infection	Others	Erythromycin is a macrolide antibiotic that has an antimicrobial spectrum similar to or slightly wider than that of penicillin (IC50=1.5 μg/ml).
Erythromycin Ethylsuccinate	Infection	Others	Erythromycin Ethylsuccinate, an oral macrolide antibiotic produced by Streptomyces erythreus, reversibly binds to the 50S ribosome of bacteria, and inhibits protein synthesis.
Escitalopram oxalate	Infection	5-HT Receptor	Vitamin D3 (Cholecalciferol) is a form of vitamin D, binds and activates a H305F/H397Y mutant vitamin D receptor (VDR) with EC50 of 300 nM.
Esmolol HCl	Cardiovascular Disease	Adrenergic Receptor	Esmolol is a cardioselective b-blocker, used to control rapid heartbeats or abnormal heart rhythms.
Esomeprazole magnesium (Nexium)	Digestive system disease	Proton Pump	Esomeprazole magnesium is a proton pump inhibitor to reduce gastric acid secretion.
Esomeprazole sodium (Nexium)	Cancer	ATPase	Esomeprazole sodium (Nexium) is a sodium salt of esomeprazole that is a potent proton pump inhibitor with an IC50 of 0.076 mg/kg.
Estradiol	Endocrinology	Estrogen/progestogen Receptor	Estradiol is the predominant sex hormone.
Estradiol Cypionate		Estrogen/progestogen Receptor	Estradiol cypionate is the 17 β-cyclopentylpropinate ester of estradiol, which inhibits ET-1 synthesis via estrogen receptor.
Estradiol valerate	Endocrinology	Estrogen/progestogen Receptor	Estradiol is a synthetic ester used to treat menopausal symptoms and hormone deficiencies.
Estriol	Neurological Disease	Estrogen/progestogen Receptor	Estriol is an antagonist of the G-protein coupled estrogen receptor in estrogen receptor-negative breast cancer cells.
Estrone	Endocrinology	Estrogen/progestogen Receptor	Estrone is an estrogenic hormone.
Ethacridine lactate monohydrate	Infection	Others	Ethacridine lactate monohydrate is an aromatic organic compound based on acridine used as an antiseptic agent.
Ethambutol HCl	Neurological Disease	Others	Ethambutol is a bacteriostatic antimycobacterial agent, which obstructs the formation of cell wall by inhibiting arabinosyl transferases.
Ethamsylate	Cardiovascular Disease	Others	Ethamsylate is a haemostatic drug, which inhibits biosynthesis and action of prostaglandins, and increases capillary endothelial resistance as and platelet adhesion.

COMPOUND	INDICATION	TARGETS	BRIEF DESCRIPTION
Ethinyl Estradiol	Endocrinology	Estrogen/progestogen Receptor	Ethynyl estradiol is an orally bio-active estrogen used in almost all modern formulations of combined oral contraceptive pills.
Ethionamide	Infection	Others	Ethionamide (2-ethylthioisonicotinamide) is an antibiotic used in the treatment of tuberculosis.
Ethoxzolamide	Neurological Disease	Others	
Ethynodiol diacetate	Endocrinology	Estrogen/progestogen Receptor	Ethynodiol diacetate is one of the first synthetic progestogens used in contraceptive pills.
Etodolac (Lodine)	Inflammation	COX	Etodolac is a nonsteroidal anti-inflammatory drug.
Etomidate	Neurological Disease	GABA Receptor	Etomidate is a GABAA receptors agonist at GABAA receptors.
Etoposide (VP-16)	Cancer	Topoisomerase	Etoposide (Etopophos) is a Topoisomerase II inhibitor (IC50 = 59.2 μM).
Etravirine (TMC125)	Neurological Disease	Reverse Transcriptase	Etravirine (TMC125) is a non-nucleoside reverse transcriptase inhibitor (NNRTI) used for the treatment of HIV.
Everolimus (RAD001)	Cancer	mTOR	Everolimus (RAD001) is an mTOR inhibitor of FKBP12 with IC50 of 1.6-2.4 nM.
Evista (Raloxifene Hydrochloride)	Endocrinology	Estrogen/progestogen Receptor	A selective estrogen receptor modulator (SERM). raloxifene inhibited the proliferation of the human breast cancer cell line, MCF-7, with IC50=0.2 nM
Exemestane	Endocrinology	Aromatase	Exemestane was found to inhibit human placental aromatase with IC50 of 42 nM.
Ezetimibe (Zetia)	Cardiovascular Disease	Others	Ezetimibe (Zetia) is a drug that lowers cholesterol.
Famciclovir (Famvir)	Cancer	Others	Famciclovir(Famvir) is a guanine analogue antiviral drug used for the treatment of various herpesvirus infections.
Famotidine (Pepcid)	Cardiovascular Disease	Histamine Receptor	Famotidine is a histamine H2–receptor antagonist with IC50 of 0.6 mM, commonly used to treat heartburn, GERD, ulcers, and other digestive conditions.
Famprofazone	Inflammation	Others	
Febuxostat (Uloric)	Inflammation	Others	Febuxostat is a non-purine selective xanthine oxidase inhibitor with IC50 of 114 -210 nM.
Felbamate	Neurological Disease	Others	Felbamate (Felbatol) is an anticonvulsant drug used in the treatment of epilepsy.

COMPOUND	INDICATION	TARGETS	BRIEF DESCRIPTION
Felodipine (Plendil)	Cardiovascular Disease	Calcium Channel	Felodipine (Plendil, Renedil) is a calcium channel blocker with an IC50 of 3 μM and 2 x 10 μM for the formyl-Met-Leu-Phe-induced cytosolic calcium increase and H2O2 production, respectively.
Fenbendazole		Others	Fenbendazole is a broad spectrum benzimidazole anthelmintic used against gastrointestinal parasites with an IC50 of about 0.01 μg/mL.
Fenofibrate (Tricor, Trilipix)	Cardiovascular Disease	PPAR	Fenofibrate (Tricor, Trilipix) is a drug of the fibrate class and fibric acid derivative
Fenoprofen calcium	Inflammation	Others	Fenoprofen calcium is a nonsteroidal, anti-inflammatory antiarthritic agent.
Fenoprofen calcium hydrate	Immunology	Others	Fenoprofen calcium hydrate (Nalfon) is a non-steroidal anti-inflammatory drug (NSAID).
Fenspiride HCl	Inflammation	PDE	Fenspiride is a bronchodilator with anti-inflammatory properties, inhibiting phosphodiesterase 4 and phosphodiesterase 3 activities with logIC50 values of 4.16 and 3.44, respectively, in human isolated bronchi.
Fenticonazole nitrate	Neurological Disease	Others	Fenticonazole nitrate is an azole antifungal agent.
Fesoterodine fumarate (Toviaz)	Immunology	AChR	Fesoterodine fumarate (Toviaz) is an antimuscarinic agent and is rapidly de-esterified to its active metabolite 5-hydroxymethyl tolterodine that is a muscarinic receptor antagonist.
Fexofenadine HCl	Neurological Disease	Histamine Receptor	Fexofenadine inhibits histamine H1 receptor with IC50 of 246 nM.
Fidaxomicin	Infection	DNA/RNA Synthesis	Fidaxomicin is a narrow spectrum macrocyclic antibiotic that inhibits RNA polymerase sigma subunit.
Finasteride	Endocrinology	5-alpha Reductase	Inhibitor of steroid Type II 5α-reductase
FK-506 (Tacrolimus)	Cancer	mTOR	An immunosuppressive agent and macrolide antibiotic.
Flavoxate HCl	Neurological Disease	AChR	Flavoxate is a muscarinic AChR antagonist with IC50 of 12.2 μM.
Florfenicol	Infection	Others	Florfenicol is a fluorinated synthetic analog of thiamphenicol with broad-spectrum, primarily bacteriostatic activity.
Floxuridine	Cancer	DNA/RNA Synthesis	Floxuridine (FUDR, FdUrd, Floxuridin) is a prodrugs of floxuridine and an oncology drug with an GI50 of 5.1 μM for the inhibition of MDCK/PEPT1.
Fluconazole	Infection	P450 (e.g. CYP17)	Fluconazole is a triazole antifungal drug used in the treatment and prevention of superficial and systemic fungal infections.
Flucytosine (Ancobon)	Infection	Others	Flucytosine (Ancobon) is a fluorinated pyrimidine analogue and a synthetic antimycotic drug.

COMPOUND	INDICATION	TARGETS	BRIEF DESCRIPTION
Fludarabine (Fludara)	Cancer	STAT,DNA/RNA Synthesis	Fludarabine (Fludara) is a purine analog and a chemotherapy drug with IC50 < 3 μM.
Fludarabine Phosphate (Fludara)	Cancer	DNA/RNA Synthesis	Fludarabine Phosphate is an analogue of adenosine and deoxyadenosine, which is able to compete with dATP for incorporation into DNA and inhibit DNA synthesis.
Flumazenil	Neurological Disease	GABA Receptor	Flumazenil is a benzodiazepine antagonist.
Flumequine	Metabolic Disease	Topoisomerase	Flumequine is a synthetic chemotherapeutic antibiotic, inhibiting topoisomerase II with IC50 of 15 μM.
Flumethasone	Endocrinology	Glucocorticoid Receptor	Flumethasone is a glucocorticoid receptor agonist, this complex binds to the nucleus causing a variety of genetic activation and repressions.
Flunarizine 2HCl	Cancer	Calcium Channel	Flunarizine dihydrochloride is a dihydrochloride salt form which is a calcium channel blocker with a Ki of 68 nM.
Flunixin meglumin	Immunology	COX	Flunixin meglumine is a potent inhibitor of the enzyme cyclooxygenase used as analgesic agent with anti-inflammatory and antipyretic activity.
Fluocinolone acetonide (Flucort-N)	Infection	Glucocorticoid Receptor	Fluocinolone(Flucort-N) acetonide is a corticosteroid that binds to the cytosolic glucocorticoid receptor.
Fluocinonide (Vanos)	Endocrinology	Glucocorticoid Receptor	Fluocinonide (Vanos) is a potent glucocorticoid steroid used topically as anti-inflammatory agent for the treatment of skin disorders such as eczema.
Fluorometholone Acetate	Inflammation	Glucocorticoid Receptor	Fluorometholone Acetate is a synthetic corticosteroid, used in the treatment of steroid responsive inflammatory conditions of the eye.
Fluoxetine HCl	Neurological Disease	5-HT Receptor	Fluoxetine (Prozac, Sarafem) is an antidepressant of the selective serotonin reuptake inhibitor (SSRI)class.
Flurbiprofen (Ansaid)	Inflammation	Others	Flurbiprofen is a member of the phenylalkanoic acid derivative family of non-steroidal anti-inflammatory drugs (NSAIDs).
Flutamide (Eulexin)	Cancer	Androgen Receptor	Flutamide (Eulexin) is an oral nonsteroidal antiandrogen agent primarily used to treat prostate cancer.
Fluticasone propionate (Flonase, Veramyst)	Inflammation	Glucocorticoid Receptor	Fluticasone propionate (Flonase, Veramyst) is a synthetic corticosteroid which is derived from fluticasone used to treat asthma and allergic rhinitis (hay fever).
Fluvastatin sodium (Lescol)	Cardiovascular Disease	HMG-CoA Reductase	Fluvastatin Sodium (Lescol) is an orally active, potent and competitive HMG-CoA reductase inhibitor with an IC50 of 70 nM for vascular smooth muscle proliferation in vitro.
Fluvoxamine maleate	Neurological Disease	5-HT Receptor	Fluvoxamine maleate is a selective serotonin (5-HT) reuptake inhibitor (SSRI)
Formoterol hemifumarate	Neurological Disease	Adrenergic Receptor	Formoterol hemifumarate is a potent, selective and long-acting β2-adrenoceptor agonist to β2 and β1 receptors with pKd of 8.12 and 5.58, respectively.

COMPOUND	INDICATION	TARGETS	BRIEF DESCRIPTION
Fosaprepitant dimeglumine	Cardiovascular Disease	Others	Fosaprepitant (MK-0517, L-758,298) is a water-soluble phosphoryl prodrug for Aprepitant which is a NK1 antagonist.
Fosfomycin Tromethamine		Others	
Ftorafur	Cancer	DNA/RNA Synthesis	Ftorafur is a substance being used in the treatment of some types of cancer.
Fulvestrant (Faslodex)	Cancer	Estrogen/progestogen Receptor	Synthetic estrogen receptor antagonist (SERD)
Furaltadone HCl	Infection	Others	Furaltadone HCl is an antibacterial and has distinct curative effect in the treatment of coccidiosis.
Furosemide (Lasix)	Cardiovascular Disease	Others	Furosemide (Lasix) is a loop diuretic used in the treatment of congestive heart failure and edema.
Gabexate mesylate	Cardiovascular Disease	Serine Protease	Gabexate is a serine protease inhibitor with IC50 of 0.19 μM which is used therapeutically in the treatment of pancreatitis and disseminated intravascular coagulation.
Gallamine triethiodide (Flaxedil)	Inflammation	AChR	Gallamine triethiodide (Flaxedil) is a cholinergic receptor blocker with an IC50 of 68.0 ± 8.4 μM.
Ganciclovir	Infection	Others	Ganciclovir is an antiviral drug for feline herpesvirus type-1 with IC50 of 5.2 μM.
Gatifloxacin		Topoisomerase	Gatifloxacin is an antibiotic of the fourth-generation fluoroquinolone family, and inhibits the bacterial enzymes DNA gyrase and topoisomerase IV.
Gefitinib (Iressa)	Cancer	EGFR	Gefitinib also known as ZD-1839 & Iressa is a novel potent EGFR tyrosine kinase & Akt phosphorylations inhibitor with IC50 of 37, 26 and 57 nM.
Gemcitabine (Gemzar)	Metabolic Disease	DNA/RNA Synthesis,Autophagy	Gemcitabine(Gemzar) belongs to the group of medicines called antimetabolites.
Gemfibrozil (Lopid)	Cardiovascular Disease	PPAR	Gemfibrozil (Lopid) is an oral drug used to lower lipid levels.
Genipin		Others	Genipin is an active aglycone derived from an iridoid glycoside called geniposide, which is found in the fruit of Gardenia jasminoides Ellis.
Geniposide		Others	Geniposide is an iridoid glycoside isolated from the fruit of Gardenia jasminoides Ellis.
Geniposidic acid		Others	Geniposidic acid is an iridoid glucoside, used to treat inflammation, jaundice and hepatic disorders.
Genistein	Cancer	EGFR,Topoisomerase	Genistein is a soy-derived isoflavone and phytoestrogen with antineoplastic activity.

COMPOUND	INDICATION	TARGETS	BRIEF DESCRIPTION
Gestodene	Endocrinology	Estrogen/progestogen Receptor	Gestodene is a progestogen hormonal contraceptive.
Gimeracil	Neurological Disease	Dehydrogenase	Gimeracil is an inhibitor of dihydropyrimidine dehydrogenase, which inhibits the early step in homologous recombination for double strand breaks repair.
Ginkgolide A	Cardiovascular Disease	GABA Receptor	Ginkgolide A is an extract from in Ginkgo biloba and a g-aminobutyric acid (GABA) antagonist with a Ki of 14.5 μM.
Glafenine HCl	Inflammation	Others	
Gliclazide (Diamicron)	Neurological Disease	Potassium Channel	Gliclazide (Diamicron) is a whole-cell beta-cell ATP-sensitive potassium currents blocker with an IC50 of 184 ± 30 nM.
Glimepiride	Metabolic Disease	Potassium Channel	Glimepiride is a medium-to-long acting sulfonylurea anti-diabetic drug.
Glipizide (Glucotrol)	Endocrinology	Others	Glipizide (Glucotrol) is used to treat high blood sugar levels caused by a type of diabetes mellitus called type 2 diabetes.
Gliquidone	Metabolic Disease	Potassium Channel	Gliquidone is an ATP-sensitive K+ channel antagonist with IC50 of 27.2 nM.
Glyburide (Diabeta)	Endocrinology	Potassium Channel	Glyburide (Diabeta) is an anti-diabetic drug in a class of medications known as sulfonylureas, closely related to sulfa drugs.
Guaifenesin (Guaiphenesin)	Respiratory Disease	Others	Guaifenesin (Guaiphenesin) is thought to act as an expectorant.
Guanabenz acetate	Endocrinology	Adrenergic Receptor	Guanabenz Acetate is an selective agonist of α2a-adrenergic receptor, α2b-adrenergic receptor and α2c-adrenergic receptor with pEC50 of 8.25, 7.01 and ~5, respectively.
Guanidine HCl	Vermifuge	Others	Guanidine HCl, the crystalline compound of strong alkalinity formed by the oxidation of guanine, is a normal product of protein metabolism and a protein denaturant.
Halobetasol Propionate	Inflammation	Phospholipase (e.g. PLA)	Halobetasol Propionate is an anti-inflammatory and a dermatologic agent commonly used to treat psoriasis.
Haloperidol (Haldol)	Neurological Disease	Others	Haloperidol (Haldol)) is an antipsychotic and butyrophenone.
Homatropine Bromide	Infection	AChR	Homatropine Bromide is muscarinic AChR antagonist, inhibits endothelial and smooth muscle muscarinic receptors of WKY-E and SHR-E with IC50 of 162.5 nM and 170.3 nM, respectively.
Homatropine Methylbromide		AChR	Homatropine Methylbromide is muscarinic AChR antagonist, inhibits endothelial and smooth muscle muscarinic receptors of WKY-E and SHR-E with IC50 of 162.5 nM and 170.3 nM, respectively.
Hydrochlorothiazide	Cardiovascular Disease	Others	Hydrochlorothiazide is a first line diuretic drug of the thiazide class.

COMPOUND	INDICATION	TARGETS	BRIEF DESCRIPTION
Hydrocortisone (Cortisol)	Infection	Glucocorticoid Receptor	Hydrocortisone is a steroid hormone or glucocorticoid produced by the adrenal gland.
Hydroxyurea (Cytodrox)	Cancer	DNA/RNA Synthesis	Hydroxyurea (Cytodrox) is an antineoplastic agent that inhibits DNA synthesis through the inhibition of ribonucleoside diphosphate reductase.
Hydroxyzine 2HCl	Neurological Disease	Histamine Receptor	Hydroxyzine is a histamine H1-receptor antagonist, inhibits binding of [3H]pyrilamine/[3H]desloratadine to human histamine H1 receptor with IC50 of 10 nM/19 nM.
Hyoscyamine (Daturine)	Neurological Disease	AChR	Hyoscyamine (Daturine) is an AChR inhibitor with IC50 of 7.5 nM.
Ibuprofen (Advil)	Inflammation	COX	Ibuprofen is a non-selective COX inhibitor with an IC50 of 3.3 x 10-4 M.
Ibutilide fumarate	Cardiovascular Disease	Sodium Channel	Ibutilide is a Class III antiarrhythmic agent that is indicated for acute cardioconversion of atrial fibrillation and atrial flutter of a recent onset to sinus rhythm by induction of slow inward sodium current, which prolongs action potential and refractory period of myocardial cells.
Idarubicin HCl	Cancer	Topoisomerase	Idarubicin is the anthracycline antibiotic and target DNA topoisomerase II (topo II). MCF-7 cells were sensitive to idarubicin, with an IC 50 value for growth inhibition of 0.01 μM
Idebenone	Inflammation	Others	Idebenone is a synthetic analog of coenzyme Q10 (CoQ10) and a brain stimulant.
Idoxuridine	Infection	Others	Idoxuridine is an antiviral agent for feline herpesvirus type-1 with IC50 of 4.3 μM.
Ifosfamide	Cancer	DNA/RNA Synthesis	Ifosfamide is a nitrogen mustard alkylating agent used in the treatment of cancer.
Iloperidone (Fanapt)	Neurological Disease	5-HT Receptor	Iloperidone (Fanapt, Fanapta, Zomaril) is an atypical antipsychotic for the treatment of schizophrenia.
Imatinib (Gleevec)	Neurological Disease	PDGFR	Imatinib is a multi-target inhibitor of v-Abl, c-Kit and PDGFR with IC50 of 0.6 μM, 0.1 μM and 0.1 μM, respectively.
Imatinib Mesylate	Cancer	c-Kit,Bcr-Abl,PDGFR	Imatinib Mesylate is a multitargeted c-kit, PDGF-R and c-ABL inhibitor with IC50 of 3.9 and 2.9 μM for the inhibition of T-cell proliferation stimulated by DCs and PHA, respectively.
Imidapril (Tanatril) HCl	Cardiovascular Disease	RAAS	Imidapril HCl is a angiotensin-converting enzyme (ACE) inhibitor with IC50 of 2.6 nM, used for the treatment of hypertension.
Imipramine HCl	Neurological Disease	Others	
Indacaterol Maleate	Infection	Adrenergic Receptor	Indacaterol is an ultra-long-acting β-adrenoceptor agonist with pKi of 7.36.
Indapamide (Lozol)	Cardiovascular Disease	Others	Indapamide (Lozol) is a non-thiazide sulphonamide diuretic drug, generally used in the treatment of hypertension, as well as decompensated cardiac failure.

COMPOUND	INDICATION	TARGETS	BRIEF DESCRIPTION
Indomethacin (Indocid, Indocin)	Inflammation	COX	Indomethacin (Indocid, Indocin) is a non-steroidal anti-inflammatory drug commonly used to reduce fever, pain, stiffness, and swelling.
Ipratropium bromide	Respiratory Disease	AChR	Ipratropium bromide is a muscarinic antagonist, bronchodilator, N-Isopropyl salt of atropine.
Irinotecan	Cancer	Topoisomerase	Irinotecan (Camptosar, Campto, CPT-11) is a topoisomerase I inhibitor with an IC50 of 15.8 and 5.17 μM for LoVo cells and HT-29 cells, respectively.
Irinotecan HCl Trihydrate (Campto)	Neurological Disease	Topoisomerase	Irinotecan prevents DNA from unwinding by inhibition of topoisomerase 1.
Irsogladine	Neurological Disease	AChR,PDE	Irsogladine is an anti-gastric ulcer agent that facilitates gap-junctional intercellular communication through M1 muscarininc acetylcholine receptor binding.
Isoconazole nitrate (Travogen)	Infection	Others	Isoconazole nitrate (Travogen) is an azole antifungal reagent.
Isoetharine Mesylate	Cardiovascular Disease	Others	
Isoniazid (Tubizid)	Infection	Others	Isoniazid (Tubizid) is a prodrug and must be activated by bacterial catalase.
Isoprenaline hydrochloride	Infection	Adrenergic Receptor	Isoprenaline hydrochloride is a beta-adrenergic agonist.
Isosorbide		Others	Isosorbide is a heterocyclic compound that is derived from glucose, used as a diuretic.
Isotretinoin	Metabolic Disease	Hydroxylase	It was developed to be used as a chemotherapy medication for the treatment of brain cancer, pancreatic cancer and more.
Isovaleramide	Neurological Disease	Dehydrogenase	Isovaleramide is an anticonvulsant molecule isolated from Valeriana pavonii, it inhibits the liver alcohol dehydrogenases.
Isoxicam	Inflammation	Others	
Isradipine (Dynacirc)	Neurological Disease	Calcium Channel	Isradipine(Dynacirc) is a calcium channel blocker with an IC50 of 34±8 μM.
Itraconazole (Sporanox)	Cancer	P450 (e.g. CYP17)	Itraconazole (Sporanox) is a triazole antifungal agent.
Ivabradine HCl (Procoralan)	Neurological Disease	Adrenergic Receptor	Ivabradine, a new If inhibitor with IC 50 of 2.9 μM which acts specifically on the pacemaker activity of the sinoatrial node, is a pure heart rate lowering agent.
Ivacaftor (VX-770)	Respiratory disease	CFTR	Ivacaftor (VX-770, Kalydeco) is a potentiator of CFTR targeting G551D-CFTR and F508del-CFTR with EC50 of 100 nM and 25 nM, respectively.

COMPOUND	INDICATION	TARGETS	BRIEF DESCRIPTION
Ivermectin	Vermifuge	Others	Ivermectin is a broad-spectrum antiparasitic medication.
Ketoconazole	Infection	P450 (e.g. CYP17)	Ketoconazole is a synthetic broad-spectrum antifungal agent.
Ketoprofen (Actron)	Inflammation	COX	Ketoprofen is a non-selective NSAID with IC50 of 0.5 μM and 2.33 μM for human recombinant COX-1 and COX-2, respectively.
Ketorolac (Toradol)	Neurological Disease	COX	Ketorolac tromethamine is a non-selective COX inhibitor with IC50 of 31.5 μM and 60.5 μM for human recombinant COX-1 and COX-2, respectively.
Ketotifen fumarate (Zaditor)	Neurological Disease	Histamine Receptor	Ketotifen fumarate (Zaditor) is a fumaric acid salt of ketotifen which is a H1-antihistamine and mast cell stabilizer.
L-Adrenaline (Epinephrine)	Cardiovascular Disease	Adrenergic Receptor	L-Adrenaline (Epinephrine) belongs to a group of the compounds known as catecholamines.
L-Thyroxine	Neurological Disease	Others	L-Thyroxine is a synthetic form of thyroxine and a hormone replacement drug.
Lacidipine (Lacipil, Motens)	Cardiovascular Disease	Calcium Channel	Lacidipine (Lacipil, Motens) is a L-type calcium channel blocker.
Lafutidine	Infection	Histamine Receptor	Lafutidine, a newly developed histamine H(2)-receptor antagonist, inhibits gastric acid secretion.
Lamivudine (Epivir)	Infection	Reverse Transcriptase	Lamivudine (Epivir) is a potent nucleoside analog reverse transcriptase inhibitor with an IC50 of 2.7 mM.
Lamotrigine	Cancer	5-HT Receptor,Sodium Channel	Lamotrigine is a novel anticonvulsant drug for inhibition of 5-HT with IC50 of 240 μM and 474 μM in human platelets and rat brain synaptosomes, respectively.
Lansoprazole	Infection	Proton Pump	Lansoprazole is a proton-pump inhibitor (PPI) which prevents the stomach from producing gastric acid.
Lapatinib	Neurological Disease	HER2,EGFR	Lapatinib, used in the form of Lapatinib Ditosylate, is a potent EGFR and ErbB2 inhibitor with IC50 of 10.8 and 9.2 nM, respectively.
Lapatinib Ditosylate (Tykerb)	Cancer	EGFR,HER2	Lapatinib Ditosylate (GW572016, GW2016) is a potent EGFR and ErbB2 inhibitor with IC50 of 10.8 and 9.2 nM, respectively.
Leflunomide	Inflammation	Dehydrogenase	Immunosuppressant agent. Its active metabolite is A77 1726 (RS-61980).
Lenalidomide	Cardiovascular Disease	TNF-alpha	Lenalidomide also known as CC-5013 & Revlimid is TNF-alpha inhibitor. Revlimid with purity >99% & solubility DMSO is available.
Letrozole	Endocrinology	Aromatase	Aromatase inhibitor. CGS 20267 is a new non-steroidal compound which potently inhibits aromatase in vitro (IC50 of 11.5 nM) and in vivo (ED50 of 1–3 μg/kg p.o.)

COMPOUND	INDICATION	TARGETS	BRIEF DESCRIPTION
Levetiracetam	Neurological Disease	Calcium Channel	Levetiracetam is an antiepileptic compound.
Levobetaxolol HCl	Cardiovascular Disease	Adrenergic Receptor	Levobetaxolol exhibits a higher affinity at cloned human β1 and β2 receptors with Ki value of 0.76 nM and 32.6 nM, respectively.
Levofloxacin (Levaquin)	Infection	Topoisomerase	Levofloxacin (Levaquin) is a synthetic fluoroquinolone (fluoroquinolones) antibacterial agent.
Levonorgestrel (Levonelle)	Endocrinology	Estrogen/progestogen Receptor	Levonorgestrel (Levonelle) is a synthetic progestogen used as an active ingredient in some hormonal contraceptives.
Levosimendan	Metabolic Disease	Others	Levosimendan is a calcium sensitizer acting through calcium-dependent binding to cardiac troponin C (cTnC), provides treatment for heart failure. Phase 4.
Levosulpiride (Levogastrol)	Neurological Disease	Dopamine Receptor	Levosulpiride is a selective antagonist for D2 dopamine receptors used as an antipsychotic and prokinetic agent.
Licofelone		COX	Licofelone is a dual COX/LOX inhibitor being considered as a treatment for osteoarthritis.
Lidocaine (Alphacaine)	Neurological Disease	Histamine Receptor	Lidocaine is a common local anesthetic and antiarrhythmic drug.
Linagliptin (BI-1356)	Cancer	DPP-4	Linagliptin is a highly potent, selective DPP-4 inhibitor with IC50 of 1 nM.
Lincomycin hydrochloride (Lincocin)	Cancer	Others	Lincomycin hydrochloride (Lincocin) is the monohydrated salt of lincomycin, a substance produced by the growth of a member of the lincolnensis group of Streptomyces lincolnensis.
Linezolid (Zyvox)	Infection	Others	Linezolid is a synthetic antibiotic used for the treatment of serious infections.
Liothyronine Sodium	Endocrinology	Others	Liothyronine Sodium is the most potent form of thyroid hormone acting on the body to increase the basal metabolic rate, affect protein synthesis.
Lithocholic acid	Neurological Disease	FXR	Lithocholic acid is a bile acid that acts as a detergent to solubilize fats for absorption.
Lomerizine HCl	Cardiovascular Disease	Calcium Channel	Lomerizine dihydrochloride is a relatively new L- and T-type calcium channel blocker used in the treatment of migraine.
Lomustine (CeeNU)	Cancer	Others	Lomustine (CeeNU) is an alkylating agent of value against both hematologic malignancies and solid tumors.
Lonidamine	Cardiovascular Disease	Others	Lonidamine is an orally administered small molecule hexokinase inactivator with an IC50 of 0.85 mM.
Loperamide hydrochloride	Infection	Autophagy,Opioid Receptor	Loperamide is an opioid-receptor agonist with an ED50 of 0.15 mg/kg.

COMPOUND	INDICATION	TARGETS	BRIEF DESCRIPTION
Lopinavir (ABT-378)	Infection	HIV Protease	Lopinavir is an inhibitor of the HIV protease.
Loratadine	Inflammation	Histamine Receptor	Loratadine (Claritin) is an antihistamine drug used to treat allergies.
Lornoxicam (Xefo)	Inflammation	COX	Lornoxicam (Xefo) is a COX-1 and COX-2 inhibitor with IC50 of 5 nM and 8 nM, respectively.
Losartan potassium	Cardiovascular Disease	RAAS	Losartan is an angiotensin II receptor antagonist, competes with the binding of angiotensin II to AT1 receptors with IC50 of 20 nM.
Loteprednol etabonate		Glucocorticoid Receptor	Loteprednol etabonate is a potent glucocorticoid receptor agonist, used in treatment of inflammation of the eye due to allergies.
Lovastatin (Mevacor)	Respiratory Disease	HMG-CoA Reductase	Lovastatin is an inhibitor of HMG-CoA reductase with IC50 of 3.4 nM, used for lowering cholesterol (hypolipidemic agent).
Loxapine Succinate	Neurological Disease	5-HT Receptor,Dopamine Receptor	Loxapine Succinate is a D2DR and D4DR inhibitor, serotonergic receptor antagonist and also a dibenzoxazepine anti-psychotic agent.
Malotilate	Metabolic Disease	Others	Malotilate is a liver protein metabolism improved compound.
Manidipine (Manyper)	Metabolic Disease	Calcium Channel	Manidipine (Manyper) is a lipophilic, third-generation, highly vasoselective dihydropyridine calcium antagonist with an IC50 of 2.6 nM.
Maprotiline hydrochloride	Neurological Disease	Adrenergic Receptor	Maprotiline hydrochloride (Deprilept, Ludiomil, Psymion) is a selective noradrenalin re-uptake inhibitor and a tetracyclic antidepressant.
Maraviroc	Inflammation	CCR	Maraviroc is a selectiveantagonist of the CCR5co-receptorwith an in vitroIC50 of 0.1 to 4.5 nMand in vitro IC90 0.6to 13.4 nM.
Marbofloxacin		Topoisomerase	Marbofloxacin is a potent antibiotic inhibiting bacterial DNA replication.
Masitinib (AB1010)	Respiratory Disease	PDGFR,c-Kit	Masitinib also known as Masivet, AB1010 is a tyrosine kinase, c-Kit, PDGFR, FGFR3, the FAK pathway inhibitor with IC50 of 150 ± 80, 200 ± 40 nM.
MDV3100 (Enzalutamide)	Cancer	Androgen Receptor	MDV3100 is an androgen-receptor (AR) antagonist with IC50 of 36 nM.
Mecarbinate	Metabolic Disease	Others	Mecarbinate is a antihypertensive agents through relaxing blood smooth muscle.
Meclofenamate Sodium		COX	Meclofenamate Sodium is a dual COX-1/COX-2 inhibitor with IC50 of 40 nM and 50 nM, respectively, used in the treatment of joint, muscular pain, arthritis and dysmenorrhea.
Medetomidine HCl	Infection	Adrenergic Receptor	Medetomidine is a selective α2-adrenoceptor agonist, with Ki of 1.08 nM, exhibts 1620-fold selectivity over α1-adrenoceptor.

COMPOUND	INDICATION	TARGETS	BRIEF DESCRIPTION
Medroxyprogesterone acetate	Infection	Estrogen/progestogen Receptor	Medroxyprogesterone acetate is a progestin, a synthetic variant of the human hormone progesterone and a potent progesterone receptor agonist.
Mefenamic acid	Cardiovascular Disease	COX	Mefenamic acid is a competitive inhibitor of COX-1 and COX-2.
Megestrol Acetate	Infection	Androgen Receptor,Estrogen/progestogen Receptor	Megestrol Acetate is a synthetic progesteronal agent with an IC50 of 260 μM for the inhibition of HegG2.
Meglumine		Others	Meglumine is an amino sugar derived from sorbitol for THP-1 cells with IC50 of 22 μg/mL.
Melatonin	Endocrinology	MT Receptor	Melatonin is a hormone produced in the brain by the pineal gland from the amino acid tryptophan.
Meloxicam (Mobic)	Inflammation	COX	Meloxicam (Mobic) is a nonsteroidal anti-inflammatory agent with analgesic and fever reducer effects.
Memantine HCl (Namenda)	Neurological Disease	AMPA Receptor-kainate Receptor-NMDA Receptor	Memantine hydrochloride (Namenda) is a CYP2B6 and CYP2D6 inhibitor for recombinant CYP2B6 and CYP2D6 with Ki of 0.51 nM and 94.9 μM, respectively.
Menadione	Endocrinology	Others	Menadione(Vitamin K3), a synthetic naphthoquinone without the isoprenoid side chain and biological activity, but can be converted to active vitamin K2, menaquinone, after alkylation in vivo.
Mepenzolate Bromide		Others	
Mepiroxol		Others	
Mepivacaine HCl	Metabolic Disease	Others	Mepivacaine is a tertiary amine used as a local anesthetic.
Meptazinol HCl		Opioid Receptor	Meptazinol is a unique centrally active opioid analgesic, which inhibits [³H]dihydromorphine binding with IC50 of 58 nM.
Mequinol	Infection	Others	Mequinol (4-Methoxyphenol) is a depigmentation agent.
Mercaptopurine	Cancer	DNA/RNA Synthesis	Mercaptopurine is a drug used to treat leukemia.
Meropenem	Infection	Others	Meropenem is an ultra-broad spectrum injectable antibiotic.
Mesalamine (Lialda)	Inflammation	IκB/IKK	Mesalamine (Lialda, Apriso) is an anti-inflammatory drug.
Mesna (Uromitexan, Mesnex)	Cancer	Others	Mesna (Uromitexan, Mesnex), a sulfhydryl compound that is used to reduce the incidence of hemorrhagic cystitis associated with certain chemotherapeutic agents.

COMPOUND	INDICATION	TARGETS	BRIEF DESCRIPTION
Mesoridazine Besylate	Neurological Disease	Others	
Mestranol	Endocrinology	Estrogen/progestogen Receptor	Mestranol is the 3-methyl ether of ethinylestradiol. It was the estrogen used in many of the first oral contraceptives.
Metaproterenol Sulfate	Respiratory Disease	Others	
Metaraminol Bitartrate		Others	
Methacycline hydrochloride (Physiomycine)	Cancer	Others	Methacycline hydrochloride (Physiomycine) is a tetracycline antibiotic.
Methazolamide	Neurological Disease	Carbonic Anhydrase	Methazolamide is a carbonic anhydrase inhibitor with Ki of 50 nM, 14 nM and 36 nM for hCA I, hCA II and bCA IV isoforms, respectively.
Methazolastone	Cancer	Autophagy	P.o. imidazotetrazine second-generation alkylating agent.
Methenamine (Mandelamine)	Inflammation	Others	Methenamine is an antibiotic used for the treatment of urinary tract infection.
Methimazole (Tapazole, Northyx)	Endocrinology	Others	Methimazole (Tapazole, Northyx) is an antithyroid medicine.
Methocarbamol (Robaxin)	Neurological Disease	Carbonic Anhydrase	Methocarbamol (Robaxin) is a central muscle relaxant used to treat skeletal muscle spasms.
Methoxsalen (Oxsoralen)	Inflammation	Others	Methoxsalen (Oxsoralen) a naturally occurring furocoumarin compound found in several species of plants, including Psoralea corylifolia, is a drug used to treat psoriasis, eczema, vitiligo and some cutaneous Lymphomas in conjunction with exposing the skin to sunlight.
Methscopolamine (Pamine)	Neurological Disease	AChR	Methscopolamine (Pamine) is a muscarinic acetylcholine receptor blocker.
Methyclothiazide	Cardiovascular Disease	Others	Methyclothiazide is a substituted benzothiadiazide, used to treat high blood pressure and fluid retention caused by various conditions including heart disease.
Methylprednisolone	Immunology	Glucocorticoid Receptor	Methylprednisolone is a synthetic corticosteroid with anti-inflammatory and immunomodulating properties.
Methylthiouracil	Infection	Others	Methylthiouracil is an antithyroid preparation.
Meticrane	Cardiovascular Disease	Others	
Metolazone (Zaroxolyn)	Cardiovascular Disease	Others	Metolazone (Zaroxolyn) is primarily used to treat congestive heart failure and high blood pressure.

COMPOUND	INDICATION	TARGETS	BRIEF DESCRIPTION
Metoprolol tartrate	Cardiovascular Disease	Adrenergic Receptor	Pefloxacin mesylate is a synthetic chemotherapeutic agent and an antibacterial agent with IC50 of 6.7 nM.
Metronidazole (Flagyl)	Infection	DNA/RNA Synthesis	Metronidazole(Flagyl)is a nitroimidazole antibiotic medication used particularly for anaerobic bacteria and protozoa.
Mevastatin	Cardiovascular Disease	HMG-CoA Reductase	Mevastatin is a competitive inhibitor of HMG-Coenzyme A (HMG-CoA) reductase with a binding affinity 10,000 times greater than the HMG-CoA substrate itself.
Mexiletine HCl	Cardiovascular Disease	Sodium Channel	Mexiletine HCl belongs to Class IB anti-arrhythmic group of medicines, inhibits sodium channels to reduce the inward sodium current.
Mezlocillin Sodium	Infection	Others	Mezlocillin sodium is a penicillin beta-lactam antibiotic used in the treatment of bacterial infections caused by susceptible, usually gram-positive, organisms.
Mianserin hydrochloride	Neurological Disease	Histamine Receptor	Mianserin is a psychoactive drug of the tetracyclic antidepressant.
Miconazole (Monistat)	Neurological Disease	Others	Miconazole (Monistat) is an imidazole antifungal agent.
Miconazole nitrate	Infection	Others	Miconazole is an imidazole antifungal agent that is used topically and by intravenous infusion.
Mifepristone (Mifeprex)	Metabolic Disease	Estrogen/progestogen Receptor	Mifepristone (Mifeprex, RU-486, RU-38486, Mifegyne) is a remarkably active antagonist of progesterone receptor and glucocorticoid receptor with IC50 of 0.2 nM and 2.6 nM, respectively.
Miglitol (Glyset)	Neurological Disease	Others	Miglitol (Glyset) is an oral anti-diabetic drug.
Milnacipran HCl	Endocrinology	Others	Milnacipran inhibits both norepinephrine transporter (NET) and norepinephrine transporter (SERT) with IC50 of 77 nM and 420 nM, respectively.
Milrinone (Primacor)	Cardiovascular Disease	ATPase,PDE	Milrinone (Primacor) is a a potent and selective phosphodiesterase 3 inhibitor with an IC50 of 0.42 μM for the inhibition of FIII PDE.
Mirabegron (YM178)	Cancer	Adrenergic Receptor	β3-adrenoceptor
Mirtazapine (Remeron, Avanza)	Immunology	5-HT Receptor	Mirtazapine (Remeron, Avanza) is a potent tetracyclic antidepressant.
Mitotane (Lysodren)	Cancer	Others	Mitotane(Lysodren), is an antineoplastic medication used in the treatment of adrenocortical carcinoma.
Mitoxantrone Hydrochloride	Cardiovascular Disease	Topoisomerase	Mitoxantrone is a type II topoisomerase inhibitor with IC50 of 2.0 μM, 0.42 mM for HepG2 and MCF-7/wt cells, respectively.
Moclobemide	Neurological Disease	MAO	Moclobemide is MAO-A (5-HT) inhibitor with IC50 of 6.1 μM.

COMPOUND	INDICATION	TARGETS	BRIEF DESCRIPTION
Moexipril HCl	Digestive system disease	RAAS	Moexipril hydrochloride is a potent orally active non-sulfhydryl angiotensin converting enzyme inhibitor (ACE) with IC50 of 0.041 μM, which is used for the treatment of hypertension and congestive heart failure.
Moguisteine	Respiratory Disease	Others	Moguisteine is a novel peripheral non-narcotic antitussive drug.
Mometasone furoate	Inflammation	Glucocorticoid Receptor	Mometasone furoate is a glucocorticosteroid used topically to reduce inflammation of the skin or in the airways.
Monobenzone (Benoquin)	Metabolic Disease	Others	Monobenzone (Benoquin) is a compound used as a topical drug for medical depigmentation.
Montelukast Sodium	Respiratory Disease	Others	Montelukast selectively antagonizes leukotriene D 4 (LTD4) by binding to it so that block the action of leukotriene D4 on the cysteinyl leukotriene receptor CysLT1.
Moroxydine	Cancer	Others	Moroxydine hydrochloride is a synthetic antiviral drug chemically belonging to the series of the heterocyclic biguanidines.
Mosapride citrate	Neurological Disease	5-HT Receptor	Mosapride is a gastroprokinetic agent that acts as a selective 5HT4 agonist.
Moxalactam Disodium	Infection	Others	
Moxifloxacin hydrochloride	Infection	Topoisomerase	Moxifloxacin (Avelox, Avalox) is a fourth generation synthetic fluoroquinolone antibacterial agent.
Moxonidine	Digestive system disease	Others	Moxonidine is a selective agonist at the imidazoline receptor subtype 1, used as antihypertensive agent.
Mupirocin		DNA/RNA Synthesis	Mupirocin is an isoleucyl t-RNA synthetase inhibitor, used of the treatment of bacterial skin infections.
Mycophenolate mofetil (CellCept)	Immunology	Dehydrogenase	Mycophenolate mofetil is a inhibitor of inosine monophosphate dehydrogenase and a immunosuppressant.
Mycophenolic (Mycophenolate)	Infection	Dehydrogenase	Mycophenolic acid (Mycophenolate) is an immunosuppressant drug used to prevent rejection in organ transplantation.
Nabumetone	Inflammation	COX	Nabumetone is a non-steroidal anti-inflammatory drug and its active metabolite inhibits the COX.
Nadifloxacin	Neurological Disease	Others	Nadifloxacin is a topical fluoroquinolone antibiotic for the treatment of acne vulgaris.
Nafamostat mesylate	Cardiovascular Disease	Serine Protease	Nafamostat is an anticoagulant.
nafcillin sodium monohydrate	Endocrinology	Others	Nafcillin sodium reversibly inhibits β-lactamase with Kd of 33 mM.

COMPOUND	INDICATION	TARGETS	BRIEF DESCRIPTION
Naftopidil (Flivas)	Endocrinology	Adrenergic Receptor	Naftopidil is a selective α1-adrenergic receptor antagonist or alpha blocker with a Ki of 58.3 nM.
Nalidixic acid (NegGram)	Infection	Topoisomerase	Nalidixic acid(NegGram) is a synthetic 1,8-naphthyridine antimicrobial agent with a limited bacteriocidal spectrum. It is an inhibitor of the A subunit of bacterial DNA gyrase.
Nalmefene HCl	Neurological Disease	Others	
Naloxone HCl	Cancer	Opioid Receptor	Naloxone HCl is an opioid inverse agonist drug used to counter the effects of opiate overdose.
Naltrexone HCl	Neurological Disease	Opioid Receptor	Naltrexone is an opioid receptor antagonist with IC50 of 8 nM used primarily in the management of alcohol dependence and opioid dependence.
Naphazoline hydrochloride (Naphcon)	Neurological Disease	Adrenergic Receptor	Naphazoline hydrochloride (Naphcon) is an ocular vasoconstrictor and imidazoline derivative sympathomimetic amine.
Naproxen (Aleve)	Inflammation	COX	Naproxen (Aleve, Anaprox) is a COX inhibitor for COX-1 and COX-2 with IC50 of 2.2 μg/mL and 1.3 μg/mL, respectively.
Naratriptan HCl	Neurological Disease	5-HT Receptor	Naratriptan (Amerge) is a triptan agent that is used for the treatment of migraine headaches.
Natamycin (Pimaricin)	Infection	Others	Natamycin (Pimaricin) is a naturally occurring antifungal agent.
Nateglinide (Starlix)	Immunology	Potassium Channel	Nateglinide (Starlix) is an insulin secretagog agent that lowers blood glucose levels by stimulating insulin secretion from the pancreas.
Nebivolol (Bystolic)	Cardiovascular Disease	Adrenergic Receptor	Nebivolol (Bystolic) is a β1 receptor blocker with an IC50 of 4.5 μM.
Nefiracetam (Translon)	Neurological Disease	GABA Receptor	Nefiracetam (Translon) is cognitive enhancer with an IC50 of approximately 150–200 μM for Ro 5-4864.
Nelarabine (Arranon)	Cancer	DNA/RNA Synthesis	Nelarabine (Arranon) performes growth inhibitory activity (IC50= 1.61 μM) against MOLT-4 cells.
Nelfinavir Mesylate		HIV Protease	Nelfinavir Mesylate is a potent HIV protease inhibitor with Ki of 2 nM.
Nepafenac	Inflammation	COX	Nepafenac is a non-steroidal anti-inflammatory drug (NSAID).
Nevirapine (Viramune)	Infection	Reverse Transcriptase	Nevirapine (Viramune) is a non-nucleoside reverse transcriptase inhibitor (NNRTI) used to treat HIV-1 infection and AIDS.
Niacin (Nicotinic acid)	Metabolic Disease	Others	Niacin (Nicotinic acid) is a water-soluble vitamin belonging to the vitamin B family.

COMPOUND	INDICATION	TARGETS	BRIEF DESCRIPTION
Nialamide	Neurological Disease	Others	
Nicardipine HCl	Neurological Disease	Calcium Channel	Nicardipine is a dihydropyridine calcium-channel blocking agent used for the treatment of vascular disorders.
Nicorandil (Ikorel)	Cardiovascular Disease	Potassium Channel	Nicorandil (Ikorel) is potassium channel activator.
Nicotinamide (Niacinamide)	Neurological Disease	Sirtuin	Nicotinamide (Niacinamide) is a water-soluble vitamin and is part of the vitamin B group.
Nicotine Ditartrate	Neurological Disease	Others	
Nifedipine (Adalat)	Cardiovascular Disease	Calcium Channel	Nifedipine(Adalat), a potent vasodilator agent with calcium antagonistic action.
Nifenazone	Inflammation	Others	
Niflumic acid	Infection	GABA Receptor,COX	Niflumic acid is an inhibitor of cyclooxygenase-2 used for joint and muscular pain.
Nifuroxazide	Infection	STAT	Nifuroxazide is a cell-permeable and orally available nitrofuran-based antidiarrheal agent that effectively suppresses the activation of cellular STAT1/3/5 transcription activity with IC50 of 3 μM against IL-6-induced STAT3 activation in U3A cells
Nilotinib (AMN-107)	Cancer	Bcr-Abl	Inhibitor of BCR-ABL, IC50 < 30nM
Nilvadipine (ARC029)	Cancer	Calcium Channel	Nilvadipine (ARC029) is a potent calcium channel blocker with an IC50 of 0.03 nM.
Nimesulide	Cardiovascular Disease	COX	Nimesulide is a relatively COX-2 selective inhibitor with IC50 of 26 μM.
Nimodipine (Nimotop)	Cardiovascular Disease	Autophagy,Calcium Channel	Nimodipine (Nimotop) is a dihydropyridine derivative and an analogue of the calcium channel blocker nifedipine, with antihypertensive activity.
Nisoldipine (Sular)	Cardiovascular Disease	Calcium Channel	Nisoldipine (Sular) is a calcium channel blocker.
Nitazoxanide (Alinia, Annita)	Vermifuge	Others	Nitazoxanide (Alinia, Annita) is a synthetic nitrothiazolyl-salicylamide derivative and an antiprotozoal agent. (IC50 for canine influenza virus ranges from 0.17 to 0.21 μM)
Nithiamide		Others	Nithiamide is a non-5-nitroimidazole drugs.
Nitrendipine	Neurological Disease	Calcium Channel,Autophagy	Nitrendipine is a dihydropyridine calcium channel blocker with an IC50 of 95 nM.

COMPOUND	INDICATION	TARGETS	BRIEF DESCRIPTION
Nitrofurazone (Nitrofural)	Infection	Others	Nitrofurazone is a topical anti-infective agent with an IC50 of 22.83 ± 1.2 μM. (Rat LD50 = 590 mg/kg)
Nizatidine	Metabolic Disease	Histamine Receptor	Nizatidine (Axid) is a histamine H2-receptor antagonist with and IC50 of 6.7 nM for AChE.
Noradrenaline bitartrate monohydrate (Levophed)	Metabolic Disease	Adrenergic Receptor	Noradrenaline bitartrate monohydrate (Levophed) is a direct alpha-adrenergic receptors stimulator.
norethindrone	Neurological Disease	Others	Norethindrone (Norethisterone) is a synthetic progestin, which mimic the actions of the endogenous ovarian hormone progesterone.
Noscapine HCl	Cancer	Others	
Novobiocin sodium (Albamycin)	Cardiovascular Disease	Topoisomerase	Novobiocin (Albamycin) is a very potent bacterial DNA gyrase and human organic anion transporters with Ki of of 14.87 ± 0.40 μM for hOAT1, 4.77 ± 1.12 μM for hOAT3, and 90.50 ± 7.50 μM for hOAT4
Nystatin (Mycostatin)	Infection	Others	Nystatin (Mycostatin) is a polyene antifungal drug to which many molds and yeasts are sensitive, including Candida spp.
Olanzapine (Zyprexa)	Infection	5-HT Receptor,Dopamine Receptor	Olanzapine (Zyprexa) is a high affinity for 5-HT2 serotonin and D2 dopamine receptor antagonist.
Olmesartan medoxomil (Benicar)	Cardiovascular Disease	RAAS	Olmesartan medoxomil (Benicar) is a compound which is hydrolyzed to olmesartan that is a selective AT1 subtype angiotensin II receptor antagonist.
Olopatadine hydrochloride (Opatanol)	Neurological Disease	Histamine Receptor	Olopatadine hydrochloride (Opatanol) is a histamine blocker and mast cell stabilizer with an IC50 of 559 μM for the release of histamine.
olsalazine sodium	Inflammation	Others	Olsalazine Sodium is a anti-inflammatory prodrug, which consists of two 5-ASA moieties linked by an azo bond.
Omeprazole (Prilosec)	Metabolic Disease	Autophagy,Proton Pump	Omeprazole (Prilosec) is a proton pump inhibitor used in the treatment of dyspepsia.
Ondansetron hydrochloride (Zofran)	Infection	5-HT Receptor	Ondansetron HCl is a serotonin 5-HT3 receptor antagonist.
Orlistat (Alli, Xenical)	Metabolic Disease	Others	Orlistat is a general lipase inhibitor with IC50 of 122 ng/ml for PL from human duodenal juice.
Ornidazole	Endocrinology	Others	Ornidazole is a 5-nitroimidazole derivative with antiprotozoal and antibacterial properties against anaerobic bacteria.
Orphenadrine citrate (Norflex)	Infection	AChR	Orphenadrine citrate is a skeletal muscle relaxant, it acts in the central nervous system to produce its muscle relaxant effects.
OSI-420 (Desmethyl Erlotinib)	Cancer	EGFR	OSI-420 (Desmethyl Erlotinib,CP-473420) is an active metabolite of erlotinib which is an orally active EGFR tyrosin kinase inhibitor with IC50 of 2 and 20 nM for the inhibition of human EGFR and EGFR autophosphorylation in tumor cells.

COMPOUND	INDICATION	TARGETS	BRIEF DESCRIPTION
Ospemifene		Estrogen/progestogen Receptor	Ospemifene is a non-hormonal selective estrogen receptor modulator (SERM), used for the treatment of dyspareunia.
Otilonium Bromide	Cardiovascular Disease	AChR	Otilonium bromide is an antimuscarinic.
Ouabain	Neurological Disease	Sodium Channel	Ouabain is a selective Na+, K+-ATPase inhibitor, binds to α2 subunit and α3 subunit with Ki of 41 nM and 15 nM, respectively.
Oxaliplatin (Eloxatin)	Cancer	DNA/RNA Synthesis	Oxaliplatin (Eloxatin) is a 1,2-diaminocyclohexane (DACH) carrier ligand-based groups of platinum antitumor agent with IC50 of median 1.3, 6.2 and 1.5 mM for SW480 DLD1, HT29, respectively.
Oxaprozin	Inflammation	COX	Oxaprozin is a non-narcotic, non-steroidal anti-inflammatory drug (NSAID) used to relieve the inflammation, swelling, stiffness, and joint pain associated with osteoarthritis and rheumatoid arthritis.
Oxcarbazepine	Neurological Disease	Sodium Channel	Oxcarbazepine is an anticonvulsant and mood stabilizing drug.
Oxeladin Citrate	Respiratory Disease	Others	
Oxethazaine	Neurological Disease	Others	
Oxfendazole	Vermifuge	Others	Oxfendazole is the sulfoxide form of fenbendazole which is a broad spectrum benzimidazole anthelmintic.
Oxybuprocaine HCl	Neurological Disease	Others	Oxybuprocaine HCl is a local anesthetic, which is used especially in ophthalmology and otolaryngology.
Oxybutynin (Ditropan)	Neurological Disease	AChR	Oxybutynin (Ditropan) is an anticholinergic medication used to relieve urinary and bladder difficulties.
Oxybutynin chloride	Neurological Disease	AChR	Oxybutynin is an anticholinergic medication used to relieve urinary and bladder difficulties.
Oxymetazoline hydrochloride	Immunology	Adrenergic Receptor	Oxymetazoline hydrochloride is an α1 and α2 adrenergic receptor agonist.
Oxytetracycline (Terramycin)	Infection	Others	Oxytetracycline (Terramycin) was the second of the broad-spectrum tetracycline group of antibiotics to be discovered.
Oxytetracycline dihydrate	Infection	Others	Oxytetracycline Dihydrate is a prescription antibiotic, iinterfering with the ability of bacteria to produce essential proteins.
Ozagrel	Cardiovascular Disease	P450 (e.g. CYP17)	Ozagrel is a potent and selective thromboxane A2 synthetase inhibitor with an IC50 of 4 nM.
Ozagrel HCl	Cardiovascular Disease	P450 (e.g. CYP17)	Ozagrel HCl is a selective thromboxane A2 synthetase enzyme inhibitors with IC50 of 11 nM, used as antiasthmatic agent.

COMPOUND	INDICATION	TARGETS	BRIEF DESCRIPTION
Paclitaxel (Taxol)	Cancer	Autophagy,Microtubule Associated	Paclitaxel also known as Taxol, Onxol, Nov-Onxol is a microtubule polymer stabilizer with an IC50 of 0.1 pM for human endothelial cells.
Paeoniflorin		Others	Paeoniflorin is a herbal constituent extracted from the root of Paeonia albiflora Pall.
Pancuronium (Pavulon)	Cardiovascular Disease	AChR	Pancuronium (Pavulon) is a competitive acetylcholine antagonist with an IC50 of 5.5 ± 0.5 nM.
Paroxetine HCl	Infection	5-HT Receptor,AChR	Paroxetine is an antidepressant drug of the SSRI type.
Pasiniazid	Infection	Others	
Pazopanib	Cardiovascular Disease	PDGFR,VEGFR,c-Kit	Pazopanib is a potent and selective multi-targeted receptor tyrosine kinase inhibitor of VEGFR1, VEGFR2, VEGFR3, PDGFR-α/β, and c-Kit.
Pazopanib HCl	Cancer	PDGFR,VEGFR,c-Kit	Pazopanib (GW786034) is a novel multi-target inhibitor of VEGFR1, VEGFR2, VEGFR3, PDGFR, FGFR, c-Kit and c-Fms with IC50 of 10 nM, 30 nM, 47 nM, 84 nM, 74 nM, 140 nM and 146 nM, respectively.
PCI-32765 (Ibrutinib)	Neurological Disease	BTK	Ibrutinib is a potent and highly selective Btk inhibitor with IC50 of 0.5 nM, modestly potent to Bmx, CSK, FGR, BRK, HCK, less potent to EGFR, Yes, ErbB2, JAK3, etc.
Pefloxacin mesylate	Infection	Others	Pefloxacin mesylate is a synthetic chemotherapeutic agent and an antibacterial agent with IC50 of 6.7 nM.
Penciclovir	Infection	Others	Penciclovir is a purine acyclic nucleoside analogue with potent antiviral activity.
Penfluridol	Neurological Disease	Dopamine Receptor	Penfluridol is a highly potent, first generation diphenylbutylpiperidine antipsychotic.
Penicillin G Sodium	Infection	Others	Penicillin G Sodium is a β-lactam antibiotic produced by Penicillin spp
Pentamidine	Infection	Others	Pentamidine is an inhibitor of PRL Phosphatases and also inhibits synthesis of DNA, RNA and protein.
Pentoxifylline		Others	
Pergolide mesylate	Neurological Disease	Dopamine Receptor	Pergolide mesylate is an antiparkinsonian agent which functions as a dopaminergic agonist.
Phenacetin	Infection	COX	Phenacetin is a non-opioid analgesic without anti-inflammatory properties.
Phenazopyridine HCl	Cardiovascular Disease	Others	Phenazopyridine HCl a local analgesic that has been used in urinary tract disorders.

COMPOUND	INDICATION	TARGETS	BRIEF DESCRIPTION
Phenformin hydrochloride	Metabolic Disease	AMPK	Phenformin hydrochloride is a hydrochloride salt of phenformin that is an anti-diabetic drug from the biguanide class.
Phenindione (Rectadione)	Cardiovascular Disease	Others	Phenindione is an anticoagulant which functions as a Vitamin K antagonist.
Pheniramine Maleate	Neurological Disease	Others	Pheniramine Maleate is an antihistamine with anticholinergic properties used to treat allergic conditions such as hay fever or urticaria.
Phenothrin		Others	
Phenoxybenzamine HCl	Endocrinology	Adrenergic Receptor	Phenoxybenzamine HCl is a non-specific, irreversible alpha antagonist with an IC50 of 550 nM.
Phentolamine mesilate	Cardiovascular Disease	Adrenergic Receptor	Phentolamine mesilate is a nonselective alpha-adrenergic antagonist with IC50 of 0.1 μM.
Phenylbutazone (Butazolidin, Butatron)	Cancer	Others	Phenylbutazone (Butazolidin, Butatron) is used as a non-steroidal anti-inflammatory drug for the treatment of chronic pain, including the symptoms of arthritis.
Phenylephrine HCl	Endocrinology	Adrenergic Receptor	Phenylephrine hydrochloride is a selective α1-adrenergic receptor agonist.
Phenytoin (Lepitoin)	Endocrinology	Sodium Channel	Phenytoin (Lepitoin; NSC 8722; Phenytek; Phenytoine; Sodanton; Zentropi) is an inactive voltage-gated sodium channel stabilizer.
Phenytoin sodium (Dilantin)	Metabolic Disease	Sodium Channel	Phenytoin sodium is an inactive voltage-gated sodium channel stabilizer.
Phthalylsulfacetamide		Others	
Pidotimod	Immunology	Others	Pidotimod is an immunostimulant.
Pilocarpine HCl	Neurological Disease	AChR	Pilocarpine HCl is a nonselective muscarinic acetylcholine receptor agonist used to produce an experimental model of epilepsy.
Pimecrolimus	Cancer	Others	Inhibitor of inflammatory cytokine secretion
Pimobendan (Vetmedin)	Cardiovascular Disease	PDE	Pimobendan (Vetmedin) is a Ca(2+) sensitizer with an IC50 of 26 μM and a selective phosphodiesterase III inhibitor with an IC50 of <1 μM.
Pimozide	Neurological Disease	Others	
Pioglitazone (Actos)	Cancer	PPAR	Pioglitazone (Actos) is a selective peroxisome proliferator-activated receptor gamma stimulator.

COMPOUND	INDICATION	TARGETS	BRIEF DESCRIPTION
Pioglitazone hydrochloride (Actos)	Metabolic Disease	P450 (e.g. CYP17)	Pioglitazone HCl is a hydrochloride salt form of pioglitazone which is a cytochrome P450 (CYP)2C8 and CYP3A4 enzymes inhibitor for CYP2C8, CYP3A4 and CYP2C9 with Ki of 1.7 μM, 11.8 μM and 32.1 μM, respectively.
Piperacillin Sodium	Infection	Others	Piperacillin is a semisynthetic, broad-spectrum, ampicillin derived ureidopenicillin antibiotic proposed for pseudomonas infections.
Piromidic Acid	Infection	Others	
Piroxicam (Feldene)	Inflammation	COX	Piroxicam (Feldene, Roxam) is a non-selective COX inhibitor with an IC50 of 6 mM.
Pitavastatin calcium (Livalo)	Cardiovascular Disease	Others	Pitavastatin calcium (Livalo) is a novel member of the medication class of statins.
Pizotifen malate	Inflammation	5-HT Receptor	Pizotifen is a benzocycloheptane based agent used for recurrent migraine headaches.
PMSF (Phenylmethylsulfonyl Fluoride)	Inflammation	Cysteine Protease,Serine Protease	PMSF (Phenylmethyl sulfonyl fluoride) is an irreversible serine/cysteine protease inhibitor.
Pomalidomide	Cancer	TNF-alpha	Pomalidomide inhibits LPS-induced TNF-α release with IC50 of 13 nM.
Ponatinib (AP24534)	Cancer	Bcr-Abl,PDGFR,VEGFR,FGFR	AP24534 is a novel, potent multi-target inhibitor of Abl, PDGFRα, VEGFR2, FGFR1 and Src with IC50 of 0.37 nM, 1.1 nM, 1.5 nM, 2.2 nM and 5.4 nM, respectively.
Posaconazole	Infection	Others	Posaconazole is a triazole antifungal drug.
Potassium iodide	Endocrinology	Others	Potassium iodide is used to treat overactive thyroid and to protect the thyroid gland from the effects of radiation from inhaled or swallowed radioactive iodine.
Pralatrexate (Folotyn)	Metabolic Disease	DHFR	Pralatrexate(Folotyn) is an antifolate, and structurally a folate analog. Its IC50 is < 300 nM in some cell lines.
Pramipexole (Mirapex)	Neurological Disease	Dopamine Receptor	Pramipexole (Mirapex) is a partial/full D2S, D2L, D3, D4 receptor agonist with a Ki of 3.9, 2.2, 0.5 and 5.1 nM for D2S, D2L, D3, D4 receptor, respectively.
Pramipexole dihydrochloride monohydrate	Neurological Disease	Dopamine Receptor	Pramipexole is a partial/full D2S, D2L, D3, D4, receptor agonist with a Ki of 3.9, 2.2, 0.5, 5.1 nM.
Pramiracetam	Endocrinology	Others	Pramiracetam is a more potent nootropic drug derived from piracetam.
Pramoxine HCl	Neurological Disease	Others	Pramoxine is a topical local anesthetic that has been shown to have antipruritic properties.
Pranlukast	Immunology	Others	Pranlukast is a selective cysteinyl leukotriene receptor antagonist.

COMPOUND	INDICATION	TARGETS	BRIEF DESCRIPTION
Pranoprofen	Inflammation	COX	Pranoprofen is a PGE2 inhibitor with IC50 of 7.5 μM.
Prasugrel (Effient)	Cardiovascular Disease	P2 Receptor	A novel platelet inhibitor
Pravastatin sodium	Metabolic Disease	HMG-CoA Reductase	Pravastatin sodium is an HMG-CoA reductase inhibitor against sterol synthesis with IC50 of 5.6 μM.
Praziquantel (Biltricide)	Vermifuge	Others	Praziquantel(Biltricide, Droncit) is an anthelmintic effective against flatworms.
Prednisolone (Hydroretrocortine)	Infection	Glucocorticoid Receptor	Prednisolone (Hydroretrocortine) is a synthetic glucocorticoid with anti-inflammatory and immunomodulating properties.
Prednisolone acetate (Omnipred)	Immunology	Glucocorticoid Receptor	Prednisolone Acetate is a synthetic corticosteroid drug that is particularly effective as an immunosuppressant agent.
Prednisone (Adasone)	Immunology	Glucocorticoid Receptor	Prednisone (Adasone) is a synthetic corticosteroid drug that is particularly effective as an immunosuppressant drug.
Pregnenolone	Neurological Disease	Estrogen/progestogen Receptor	Pregnenolone is an endogenous steroid hormone for inhibition of M1 receptor- and M3 receptor-mediated currents with IC50 of 11.4 μM and 6.0 μM, respectively.
Pridinol Methanesulfonate		Others	
Prilocaine	Neurological Disease	Others	Prilocaine is a local anesthetic of the amino amide type.
Primaquine Diphosphate		Others	Primaquine Diphosphate is a transmission-blocking anti-malarial clinically available, displaying a marked activity against gametocytes of all species of human malaria.
Primidone (Mysoline)	Neurological Disease	Sodium Channel	Primidone (Mysoline) is an anticonvulsant of the pyrimidinedione class.
Proadifen HCl		Others	
Probenecid (Benemid)	Metabolic Disease	TRPV	Probenecid (Benemid) is a classical competitive inhibitor of organic anion transport, which is also a TRPV2 agonist and an inhibitor of TAS2R16.
Probucol	Cardiovascular Disease	Others	Probucol is an anti-hyperlipidemic drug by lowering the level of cholesterol in the bloodstream by increasing the rate of LDL catabolism.
Procaine (Novocaine) HCl	Neurological Disease	Sodium Channel	Procaine (Novocaine) HCl is an inhibitor of sodium channel, NMDA receptor and nAChR with IC50 of 60 μM, 0.296 mM and 45.5 μM, which is also an inhibitor of 5-HT3 with KD of 1.7 μM.
Prochlorperazine Dimaleate	Neurological Disease	Others	

COMPOUND	INDICATION	TARGETS	BRIEF DESCRIPTION
Procodazole		Others	
Procyclidine HCl	Neurological Disease	Others	
Progesterone (Prometrium)	Endocrinology	Estrogen/progestogen Receptor	Progesterone (Prometrium) is a C-21 steroid hormone.
Propafenone (Rytmonorm)	Cardiovascular Disease	Sodium Channel	Propafenone(Rytmonorm) is a classic anti-arrhythmic medication, which treats illnesses associated with rapid heartbeats such as atrial and ventricular arrhythmias.
Proparacaine HCl	Neurological Disease	Sodium Channel	Proparacaine HCl is a voltage-gated sodium channels antagonist with ED50 of 3.4 mM.
Propranolol HCl	Cardiovascular Disease	Adrenergic Receptor	Propranolol HCl is a competitive non-selective beta-adrenergic receptors inhibitor with IC50 of 12 nM.
Propylthiouracil	Endocrinology	Others	Propylthiouracil is a thyroperoxidase and 5'-deiodinase inhibitor.
Protionamide (Prothionamide)	Infection	Others	Protionamide (Prothionamide) is a drug used in the treatment of tuberculosis.
Pyrazinamide (Pyrazinoic acid amide)	Infection	Others	Pyrazinamide (Pyrazinoic acid amide) is a drug used to treat tuberculosis.
Pyridostigmine Bromide (Mestinon)	Cardiovascular Disease	AChR	Pyridostigmine Bromide (Mestinon) is a parasympathomimetic and a reversible cholinesterase inhibitor.
Pyridoxine hydrochloride	Endocrinology	Others	Pyridoxine HCl is a form of vitamin B6.
Pyrilamine Maleate	Neurological Disease	Others	
Pyrimethamine	Immunology	DHFR	Pyrimethamine is a dihydrofolate reductase(DHFR) inhibitor with an IC50 of 15.4 nM.
Pyrithione zinc	Infection	Proton Pump	Zinc pyrithione is an antifungal and antibacterial agent disrupting membrane transport by blocking the proton pump.
Quetiapine fumarate (Seroquel)	Neurological Disease	Dopamine Receptor	Quetiapine fumarate(Seroquel) is an atypical antipsychotic used in the treatment of schizophrenia, bipolar I mania, bipolar II depression, bipolar I depression.
Quinapril HCl (Accupril)	Inflammation	RAAS	Quinapril hydrochloride (Accupril) is the hydrochloride salt of quinapril that is an angiotensin-converting enzyme inhibitor with a Ki of 20 μM.
Quinine hydrochloride dihydrate	Cardiovascular Disease	Potassium Channel	Quinine hydrochloride dihydrate is a natural white crystalline alkaloid having antipyretic (fever-reducing), antimalarial, analgesic (painkilling), anti-inflammatory properties and a bitter taste.

COMPOUND	INDICATION	TARGETS	BRIEF DESCRIPTION
Racecadotril (Acetorphan)	Infection	Opioid Receptor	Racecadotril is a peripherally acting enkephalinase inhibitor with an IC50 of 4.5 μM.
Ractopamine HCl	Neurological Disease	Others	
Raltegravir (MK-0518)	Immunology	Integrase	Raltegravir (MK-0518) is anHIV integrase inhibitor, IC95 for HIV-1 in 50% normal human serum = 33 nM.
Ramelteon (TAK-375)	Neurological Disease	MT Receptor	Ramelteon is a melatonin receptor agonist with both high affinity for melatonin MT1 (IC50 =28.5 ± 8.55 pM)and MT2 (20.1 ± 9.25 pM)receptors and selectivity over the MT3 receptor.
Ramipril (Altace)	Cardiovascular Disease	RAAS	Ramipril (Altace) is an angiotensin-converting enzyme (ACE) inhibitor with an IC50 of 5 nM.
Ranitidine (Zantac)	Metabolic Disease	Histamine Receptor	Ranitidine hydrochloride (Zantac) is a histamine H2-receptor antagonist with IC50 of 3.3 ± 1.4 μM.
Ranolazine (Ranexa)	Cardiovascular Disease	Calcium Channel	Ranolazine (Ranexa) is an antianginal medication.
Ranolazine dihydrochloride	Cardiovascular Disease	Calcium Channel	Ranolazine 2HCl, is an antianginal medication.
Rapamycin (Sirolimus)	Immunology	mTOR,Autophagy	Rapamycin also known as Sirolimus & Rapamune is a mTOR inhibitor. Rapamycin Sirolimus inhibits cell motility by suppression of mTOR-mediated pathways.
Rasagiline mesylate	Cardiovascular Disease	MAO	Rasagiline mesylate is a new MAO-B inhibitor for the treatment of idiopathic Parkinson's disease.
Rebamipide	Infection	Others	Rebamipide is a cholecystokinin type 1 (CCK1) receptor inhibitor for inhibiting [125I]BH-CCK-8S with IC50 of 37.7 μM.
Reboxetine mesylate	Neurological Disease	Others	Reboxetine is a norepinephrine reuptake inhibitor with Ki of 8.2 nM.
Regorafenib (BAY 73-4506)	Cancer	c-RET,VEGFR	Regorafenib (BAY 73-4506, Fluoro-Sorafenib) is a multi-target inhibitor for VEGFR1, VEGFR2, VEGFR3, PDGFRβ, Kit, RET and Raf-1 with IC50 of 13 nM/4.2 nM/46 nM, 22 nM, 7 nM, 1.5 nM and 2.5 nM, respectively.
Repaglinide	Endocrinology	Potassium Channel	Repaglinide is for the treatment of type II diabetes.
Reserpine	Cardiovascular Disease	Others	Reserpine is an indole alkaloid antipsychotic and antihypertensive drug that has been used for the control of high blood pressure and for the relief of psychotic symptoms.
Resveratrol	Infection	Sirtuin,Autophagy	Resveratrol is a phytoalexin produced naturally by several plants with anti-cancer, anti-inflammatory, blood-sugar-lowering and other beneficial cardiovascular effects .
Retapamulin	Neurological Disease	Others	Retapamulin is a topical antibiotic, which binds to both E. coli and S. aureus ribosomes with similar potencies with Kd of 3 nM.

COMPOUND	INDICATION	TARGETS	BRIEF DESCRIPTION
Ribavirin (Copegus)	Metabolic Disease	Others	Ribavirin is an anti-viral agent indicated for severe RSV infection (individually), hepatitis C infection and other viral infections.
Rifabutin (Mycobutin)	Infection	Others	Rifabutin (Mycobutin) is a semisynthetic ansamycin antibiotic with potent antimycobacterial properties.
Rifampin (Rifadin, Rimactane)	Infection	DNA/RNA Synthesis	Rifampin (Rifadin, Rimactane) is a bactericidal antibiotic agent of the rifamycin group.
Rifapentine (Priftin)	Infection	Others	Rifapentine (Priftin) is an antibiotic drug used in the treatment of tuberculosis.
Rifaximin (Xifaxan)	Infection	DNA/RNA Synthesis	Rifaximin (Xifaxan), an orally administered, semi-synthetic, nonsystemic antibiotic derived from rifamycin SV with antibacterial activity.
Riluzole (Rilutek)	Neurological Disease	Sodium Channel,GluR	Riluzole (Rilutek) is a drug used to treat amyotrophic lateral sclerosis.
Rimantadine (Flumadine)	Infection	Others	Rimantadine (Flumadine) is an anti-influenza virus drug for T. brucei with IC50 of 7 μM.
Rimonabant (SR141716)	Inflammation	Cannabinoid Receptor	Rimonabant is a selective antagonist of CB1 with IC50 of 13.6 nM and EC50 of 17.3 nM in hCB1 transfected HEK 293 membrane.
Risperidone (Risperdal)	Neurological Disease	5-HT Receptor	Risperidone (Risperdal) is an atypical antipsychotic used to treat schizophrenia.
Ritodrine hydrochloride (Yutopar)	Infection	Adrenergic Receptor	Ritodrine hydrochloride (DU 21220; Miolene; NSC 291565; Pre-Par) is a hydrochloride salt of ritodrine which is a β-2 adrenergic receptor agonist.
Ritonavir	Infection	HIV Protease	Ritonavir is an inhibitor of HIV protease used to treat HIV infection and AIDS.
Rivaroxaban (Xarelto)	Metabolic Disease	Factor Xa	Rivaroxaban is a direct inhibitor of Factor Xa with Ki and IC50 of 0.4 nM and 0.7 nM, respectively.
Rivastigmine tartrate (Exelon)	Cardiovascular Disease	AChR	Rivastigmine, a cholinesterase inhibitor with IC50 of 5.5 μM, ues as a parasympathomimetic or cholinergic agent for the treatment of mild to moderate Alzheimer disease.
Rizatriptan Benzoate		5-HT Receptor	Rizatriptan Benzoate is an agonist at serotonin 5-HT1B and 5-HT1D receptors, used to treat acute migraine attacks.
Rocuronium bromide	Neurological Disease	AChR	Rocuronium is an aminosteroid non-depolarizing neuromuscular blocker or muscle relaxant.
Rofecoxib (Vioxx)	Digestive system disease	COX	Rofecoxib (Vioxx) is a COX-2 inhibitor with IC50 of 18 nM.
Roflumilast (Daxas)	Neurological Disease	PDE	Roflumilast(Daxas) is a selective, long-acting the enzyme PDE-4 inhibitor with an IC50 of 0.8 nM.

COMPOUND	INDICATION	TARGETS	BRIEF DESCRIPTION
Rolipram	Inflammation	PDE	Rolipram is a PDE4-inhibitor and an anti-inflammatory agent.
Ronidazole	Neurological Disease	Others	Ronidazole is an antiprotozoal agent.
Ropinirole HCl	Neurological Disease	Dopamine Receptor	Ropinirole a selective dopamine D2 receptors inhibitor with IC50 of 29 nM.
Ropivacaine HCl	Infection	Others	Ropivacaine HCl is an anaesthetic agent and blocks impulse conduction in nerve fibres through inhibiting sodium ion influx reversibly.
Rosiglitazone (Avandia)	Cancer	PPAR	Rosiglitazone (Avandia) is a potent antihyperglycemic agent and a potent thiazolidinedione insulin sensitizer with IC50 of 12, 4 and 9 nM for rat, 3T3-L1 and human adipocytes, respectively.
Rosiglitazone HCl	Cardiovascular Disease	PPAR	Rosiglitazone HCl is a blood glucose-lowering drugs, stimulating insulin secretion by binding to the PPAR receptors in fat cells.
Rosiglitazone maleate	Infection	PPAR	Rosiglitazone, a member of the thiazolidinedione class of antihyperglycaemic agents, is a high-affinity selective agonist of the peroxisome proliferator-activated receptor-γ.
Rosuvastatin calcium (Crestor)	Infection	HMG-CoA Reductase	Rosuvastatin (Crestor) is a member of statins and used to treat high cholesterol and related conditions, and to prevent cardiovascular disease.
Rotigotine		Dopamine Receptor	Rotigotine is a dopamine receptor agonist, used in the treatment of Parkinson's disease and restless legs syndrome.
Roxatidine acetate HCl	Digestive system disease	Histamine Receptor	Roxatidine Acetate HCl is a specific and competitive histamin H2-receptor antagonist, with IC50 of 3.2 μM, inhibits gastric acid secretion and ulcer formation.
Roxithromycin (Roxl-150)	Metabolic Disease	Others	Roxithromycin(Roxl-150) is a semi-synthetic macrolide antibiotic. It is used to treat respiratory tract, urinary and soft tissue infections.
Rufinamide (Banzel)	Neurological Disease	Sodium Channel	Rufinamide, a triazole derivative, is an anticonvulsant medication.
Ruxolitinib (INCB018424)	Cancer	JAK	INCB018424 is a JAK family inhibitor for JAK1, JAK2 and JAK3 with IC50 of 2.7 nM, 4.5 nM and 322 nM, respectively.
S-(+)-Rolipram	Cardiovascular Disease	PDE	S-(+)-Rolipram is a less active enantiomer of the PDE4 inhibitor with an EC50 of 1.0 μM.
Salicylanilide	Infection	Reverse Transcriptase	Salicylanilides are a group of compounds with a wide range of biological activities including antiviral potency, antibacterial (including antimycobacterial) and antifungal activities.
Sasapyrine	Inflammation	Others	Sasapyrine (salsalate) is a nonsteroidal oral anti-inflammatory agent.
Saxagliptin (BMS-477118,Onglyza)	Infection	DPP-4	Saxagliptin (BMS-477118, Onglyza) is a selective and reversible DPP4 inhibitor with IC50 of 26 nM.

COMPOUND	INDICATION	TARGETS	BRIEF DESCRIPTION
Scopine	Metabolic Disease	Adrenergic Receptor	Scopine is the metabolite of anisodine, which is a α1-adrenergic receptor agonist and used in the treatment of acute circulatory shock.
Scopolamine hydrobromide	Respiratory Disease	AChR	Scopolamine is a competitive muscarinic acetylcholine receptor with an IC50 of 55.3 ± 4.3 nM.
Secnidazole (Flagentyl)	Infection	Others	Secnidazole (Flagentyl) is a nitroimidazole anti-infective.
Serotonin HCl	Neurological Disease	5-HT Receptor	Serotonin HCl is a monoamine neurotransmitter and Endogenous 5-HT receptor agonist.
Sertaconazole nitrate	Infection	Others	Sertaconazole nitrate is a topical broad-spectrum antifungal that is developed to provide an additional agent for the treatment of superficial cutaneous and mucosal infections.
Sertraline HCl	Inflammation	5-HT Receptor	Sertraline HCl is a 5-HT antagonist with Ki of 13 nM.
Sildenafil citrate	Cardiovascular Disease	PDE	Sildenafil citrate is a drug used to treat erectile dysfunction and pulmonary arterial hypertension (PAH).
Silodosin (Rapaflo)	Cardiovascular Disease	Adrenergic Receptor	Silodosin(Rapaflo) is an α1-adrenoceptor antagonist with high uroselectivity.
Simvastatin (Zocor)	Cardiovascular Disease	HMG-CoA Reductase	Simvastatin(Zocor) is a hypolipidemic drug belonging to the class of pharmaceuticals called "statins".
Sitafloxacin Hydrate		Others	Sitafloxacin Hydrate is a new-generation, broad-spectrum oral fluoroquinolone antibiotic.
Sodium 4-aminohippurate Hydrate		Others	
Sodium ascorbate	Endocrinology	Others	Sodium Ascorbate is a more bioavailable form of vitamin C that is an alternative to taking ascorbic acid as a supplement.
Sodium nitrite	Neurological Disease	Others	Sodium nitrite is a myeloperoxidase inhibitor with IC50 of 1.3 μM.
Sodium Nitroprusside	Cardiovascular Disease	Others	Sodium Nitroprusside is a potent vasodilator working through releasing NO spontaneously in blood.
Sodium Picosulfate	Metabolic Disease	Others	Sodium Picosulfate inhibits absorption of water and electrolytes, and increases their secretion.
Sodium salicylate	Infection	NF-κB	Sodium salicylate is used in medicine as an analgesic and antipyretic.
Solifenacin succinate	Cardiovascular Disease	AChR	Solifenacin succinate is a urinary antispasmodic of the antimuscarinic class.

COMPOUND	INDICATION	TARGETS	BRIEF DESCRIPTION
Sorafenib (Nexavar)	Cancer	VEGFR,Raf,PDGFR	Sorafenib Tosylate is a novel, small molecular inhibitor of several tyrosine protein kinases (VEGFR and PDGFR) and RAF/MEK/ERK cascade inhibitor with an IC50 of 6, 22, 38 nM for Raf-1, wt BRAF and V599E mutant BRAF.
Sorbitol (Glucitol)	Digestive system disease	Others	Sorbitol(Glucitol) is a sugar alcohol and a sugar substitute.
Sotalol (Betapace)	Neurological Disease	Adrenergic Receptor	Sotalol (Betapace) is a non-selective beta blocker and a potassium channel blocker with an IC50 of 43 μM.
Sparfloxacin	Infection	Others	Sparfloxacin is a fluoroquinolone antibiotic, shows broad and potent antibacterial activity.
Spectinomycin hydrochloride	Cardiovascular Disease	Others	Spectinomycin hydrochloride is a new parenteral antibiotic prepared from Streptomyces spectabilis.
Spiramycin	Infection	Others	Spiramycin is a 16-membered ring macrolide (antibiotic).
Spironolactone	Infection	Androgen Receptor	Spironolactone is a potent antagonist of the androgen receptor with IC50 of 77 nM.
Stavudine	Infection	Reverse Transcriptase	Stavudine is a nucleoside analog reverse transcriptase inhibitor (NARTI) active against HIV.
Streptozotocin (Zanosar)	Cancer	Others	Streptozotocin (Streptozocin, Zanosar, STZ) is a naturally occurring chemical used in cancer chemotherapy and Type 1 diabetes treatment.
Sucralose		Others	Sucralose is an artificial and noncaloric sweetener, not broken down by the body.
Sulbactam	Infection	Others	Sulbactam is a beta-lactamase inhibitor with an average IC50 of 0.8 μM.
Sulbactam sodium (Unasyn)	Infection	Others	Sulbactam sodium (Unasyn) is an irreversible β-lactamase inhibitor.
Sulconazole Nitrate	Infection	Others	Sulconazole Nitrate is an imidazole derivative with broad-spectrum antifungal activity.
sulfacetamide sodium	Cardiovascular Disease	Autophagy	Sulfacetamide Sodium is an anti-biotic.
Sulfadiazine	Infection	Others	Sulfadiazine is a sulfonamide antibiotic.
Sulfaguanidine	Infection	Others	Sulfaguanidine is a sulfonamide used as an anti-infective agent.
Sulfamerazine	Infection	Others	Sulfamerazine is a sulfonamide antibacterial.

COMPOUND	INDICATION	TARGETS	BRIEF DESCRIPTION
Sulfameter (Bayrena)	Infection	DHFR	Sulfameter (Bayrena) is a long-acting sulfonamide antibacterial.
Sulfamethazine	Endocrinology	Others	Sulfamethazine is a sulfonamide antibacterial.
Sulfamethizole (Proklar)	Infection	Others	Sulfamethizole(Proklar) is a sulfathiazole antibacterial agent.
Sulfamethoxazole	Infection	Others	Sulfamethoxazole (Gantanol) is a sulfonamide bacteriostatic antibiotic with an IC50 of 2.7 μM.
Sulfanilamide	Infection	Others	Sulfanilamide is a sulfonamide antibacterial.
Sulfasalazine (Azulfidine)	Inflammation	Others	Sulfasalazine (Azulfidine) is a sulfa agent and a derivative of mesalazine used primarily as an anti-inflammatory agent.
Sulfathiazole	Infection	Others	Sulfathiazole is an organosulfur compound that has been used as a short-acting sulfa drug.
Sulfisoxazole	Infection	Others	Sulfisoxazole is a sulfonamide antibacterial with an oxazole substituent.
Sulindac (Clinoril)	Cancer	COX	Sulindac (Clinoril) is a non-steroidal anti-inflammatory drug of the arylalkanoic acid class.
Sulphadimethoxine	Infection	Others	Sulphadimethoxine is a non-reducing glucuronide.
Sumatriptan succinate	Neurological Disease	5-HT Receptor	Sumatriptan succinate (Imitrex, Imigran) is a selective 5-hydroxytryptamine1 receptor subtype agonist.
Sunitinib Malate (Sutent)	Cancer	VEGFR,PDGFR,c-Kit	Sunitinib Malate (Sutent) is a multitargeted FLT3, PDGFRs, VEGFRs, and Kit kinase inhibitor with Ki of 0.009 and 0.008 μM for Flk-1 and PDGFR.
Suplatast tosylate	Cardiovascular Disease	Others	Suplatast tosylate is a Th2 cytokine inhibitor which is used as an anti-allergic agent.
Suprofen (Profenal)	Inflammation	Others,COX	Suprofen(Profenal) is an NSAID.
Tacrine HCl	Neurological Disease	Others	
Tadalafil (Cialis)	Cardiovascular Disease	PDE	Tadalafil (Cialis) is a PDE inhibitor.
TAME	Cancer	APC,E3 Ligase	Tosyl-L-Arginine Methyl Ester (TAME) inhibits an E3 ubiquitin ligase called "anaphase-promoting complex/cyclosome (APC/C)".

COMPOUND	INDICATION	TARGETS	BRIEF DESCRIPTION
Tamoxifen Citrate (Nolvadex)	Endocrinology	Autophagy,Estrogen/progestogen Receptor	Tamoxifen (Nolvadex) is an estrogen receptor antagonist with an IC50 of 31 μM for the MCF-7 cells.
Tazarotene (Avage)	Inflammation	Others	Tazarotene is a prescription topical retinoid sold as a cream or gel.
Tebipenem pivoxil (L-084)	Cardiovascular Disease	Others	Tebipenem pivoxil(L-084) is a novel oral carbapenem antibiotic with an IC50 of 100 μg/ml for human CYP isoforms
Telaprevir (VX-950)	Infection	HCV Protease	Telaprevir (VX-950) is an HCV NS3-4A serine protease inhibitor with IC50 of 0.35 μM.
Telbivudine (Sebivo, Tyzeka)	Infection	Reverse Transcriptase	Telbivudine (Tyzeka, Sebivo) is an antiviral drug used in the treatment of hepatitis B infection.
Telmisartan (Micardis)	Cardiovascular Disease	RAAS	Telmisartan (Micardis) is an angiotensin II receptor antagonist (ARB) used in the management of hypertension.
Temocapril HCl	Cancer	RAAS	Temocapril HCl is the hydrochloride of Temocapril, which is a long-acting angiotensin-converting enzyme (ACE) inhibitor, used for the treatment of hypertension.
Temsirolimus (Torisel)	Cancer	mTOR	mTOR inhibitor.
Teniposide (Vumon)	Cancer	Topoisomerase	Teniposide (Vumon)is a chemotherapeutic medication mainly used in the treatment of childhood acute lymphocytic leukemia (ALL).
Tenofovir		Reverse Transcriptase	Tenofovir blocks reverse transcriptase and hepatitis B virus infections.
Tenofovir Disoproxil Fumarate		Reverse Transcriptase	Tenofovir Disoproxil Fumarate belongs to a class of antiretroviral drugs, it inhibits the activity of HIV reverse transcriptase by competing with the natural substrate deoxyadenosine 5'-triphosphate and, after incorporation into DNA, by DNA chain termination.
Tenoxicam (Mobiflex)	Infection	Others	Tenoxicam(Mobiflex) is a good HO. radicals scavenger with an IC50 of 56.7 μM
Terazosin HCl (Hytrin)	Infection	Adrenergic Receptor	Hytrin (terazosin) is an alpha-adrenergic blocker used to treat high blood pressure and enlarged prostate.
Terbinafine (Lamisil, Terbinex)	Infection	Others	Terbinafine (Lamisil, Terbinex) is used to treat infections caused by a fungus. It works by killing the fungus or preventing its growth.
Terbinafine hydrochloride (Lamisil)	Infection	Others	Terbinafine hydrochloride (Lamisil) is a hydrochloride salt of terbinafine that is a synthetic allylamine antifungal and a squalene epoxidase inhibitor with an IC50 of 30 nM for Candida albicans.
Terfenadine	Neurological Disease	Others	
Teriflunomide	Immunology	Dehydrogenase	Teriflunomide is the active metabolite of leflunomide, inhibiting pyrimidine de novo synthesis by blocking the enzyme dihydroorotate dehydrogenase, used as an immunomodulatory agent.

COMPOUND	INDICATION	TARGETS	BRIEF DESCRIPTION
Tetracaine hydrochloride (Pontocaine)	Endocrinology	Calcium Channel	Tetracaine hydrochloride (Pontocaine) is a hydrochloride salt form of tetracaine which is a potent local anaesthetic and a channel function allosteric inhibitor.
Tetracycline HCl	Infection	Others	Tetracycline hydrochloride is a hydrochloride salt of tetracycline that is a broad-spectrum polyketide antibiotic.
tetrahydrozoline hydrochloride	Inflammation	Adrenergic Receptor	Tetrahydrozoline HCl is an imidazoline derivative with alpha receptor agonist activity.
Thalidomide	Immunology	E3 Ligase ,TNF-alpha	Thalidomide was introduced as a sedative drug,immunomodulatory agent and also is investigated for treating symptoms of many cancers.
Thiabendazole	Vermifuge	Others	Thiabendazole inhibites the mitochondrial helminth-specific enzyme, fumarate reductase, with anthelminthic property.
Thiamphenicol (Thiophenicol)	Cardiovascular Disease	Others	Thiamphenicol (Thiophenicol) is an antimicrobial antibiotic and a methyl-sulfonyl analogue of chloramphenicol.
Thioridazine HCl	Neurological Disease	Others	
Tianeptine sodium	Neurological Disease	5-HT Receptor	Tianeptine is a selective serotonin reuptake enhancer (SSRE) compound used for treating major depressive episodes.
Ticagrelor	Cardiovascular Disease	P2 Receptor	Ticagrelor is the first reversibly binding oral P2Y12 receptor antagonist, also inhibits CYP2C9 and 4-hydroxylation with IC50 of 10.5 μM and 8.2 μM respectively.
Tigecycline	Infection	Others	Tigecycline is a glycylcycline antibiotic.
Tilmicosin	Infection	Others	Tilmicosin is a macrolide antibiotic.
tinidazole	Infection	Others	Tinidazole is an anti-parasitic drug.
Tioconazole	Infection	Others	Tioconazole is an antifungal medication with an average IC50 of 1.7 μM.
Tiopronin (Thiola)	Cardiovascular Disease	Others	Tiopronin is a US Food and Drug Administration (FDA)-approved drug for the treatment of cystinuria by controlling the rate of cystine precipitation and excretion.
Tiotropium Bromide hydrate	Infection	AChR	Tiotropium Bromide hydrate is a monohydrate of tiotropium bromide (Spiriva; Tiova; BA 679BR; tiopropium) that is an anticholinergic and bronchodilator and a muscarinic receptor antagonist.
Tioxolone	Endocrinology	Carbonic Anhydrase	Tioxolone is a metalloenzyme carbonic anhydrase I inhibitor with a Ki of 91 nM.
Tiratricol	Endocrinology	Others	Tiratricol (also known as TRIAC or triiodothyroacetic acid) is a thyroid hormone analogue.

COMPOUND	INDICATION	TARGETS	BRIEF DESCRIPTION
Tizanidine HCl	Neurological Disease	Adrenergic Receptor	Tizanidine is a drug that is used as a muscle relaxant.
Tofacitinib citrate (CP-690550 citrate)	Cancer	JAK	Tofacitinib citrate (CP-690550 citrate) is a novel inhibitor of JAK3 with IC50 of 1 nM, 20- to 100-fold less potent against JAK2 and JAK1. Phase 3.
Tolbutamide	Cancer	Potassium Channel	Tolbutamide is an inhibitor of cAMP with IC50 of 4 mM.
Tolcapone	Metabolic Disease	Transferase	Tolcapone is a selective, potent and reversible of catechol-O-methyl transferase (COMT) inhibitor with Ki of 30 nM.
Tolfenamic acid	Inflammation	COX	Tolfenamic acid is a COX-2 inhibitor with IC50 of 0.2 μM.
Tolmetin Sodium	Inflammation	Others	
Tolnaftate	Cancer	Others	Tolnaftate is a synthetic thiocarbamate used as an anti-fungal agent.
Tolperisone HCl	Neurological Disease	Sodium Channel	Tolperisone HCl is an ion channel blocker and centrally-acting muscle relaxant.
Tolterodine tartrate (Detrol LA)	Neurological Disease	AChR	Tolterodine tartrate (Detrol LA) is a tartrate salt of tolterodine that is a competitive muscarinic receptor antagonist.
toltrazuril	Infection	Others	Toltrazuril is an antiprotozoal agent that acts upon Coccidia parasites.
Tolvaptan (OPC-41061)	Metabolic Disease	Vasopressin Receptor	Tolvaptan (OPC-41061) is a selective, competitive arginine vasopressin receptor 2 antagonist with an IC50 of 1.28¦ÌM for the inhibition of AVP-induced platelet aggregation.
Topiramate	Neurological Disease	Carbonic Anhydrase	Topiramate (Topamax) is an anticonvulsant drug.
Topotecan HCl	Cancer	Topoisomerase	Topotecan Hydrochloride (Hycamtin) is a topoisomerase I inhibitor with an IC50 of 13 and 2 nM for MCF-7 Luc cells and DU-145 Luc cells.
Toremifene Citrate (Fareston, Acapodene)	Endocrinology	Estrogen/progestogen Receptor	Toremifene Citrate (Fareston, Acapodene) is an oral selective estrogen receptor modulator (SERM) which helps oppose the actions of estrogen in the body.
Tranilast (SB 252218)	Respiratory Disease	Others	Tranilast (Rizaben) is an antiallergic drug.
Tretinoin (Aberela)	Cancer	Retinoid Receptor	Tretinoin (Aberela) is a drug commonly used to treat acne vulgaris and keratosis pilaris.
Triamcinolone (Aristocort)	Inflammation	Glucocorticoid Receptor	Triamcinolone (Aristocort) is a glucocorticoid given, as the free alcohol or in esterified form, orally, intramuscularly, by local injection, by inhalation, or applied topically in the management of various disorders in which corticosteroids are indicated.

COMPOUND	INDICATION	TARGETS	BRIEF DESCRIPTION
Triamcinolone Acetonide	Inflammation	Glucocorticoid Receptor	Triamcinolone Acetonide is a more potent type of triamcinolone, being about 8 times as effective as prednisone.
triamterene	Inflammation	Sodium Channel	Triamterene blocks epithelial Na+ channel (ENaC) in a voltage-dependent manner with IC50 of 4.5 μM.
Trichlormethiazide (Achletin)	Cardiovascular Disease	Others	Trichlormethiazide(Achletin) is a diuretic with properties similar to those of hydrochlorothiazide.
Triclabendazole	Vermifuge	Microtubule Associated	Triclabendazole is a benzimidazole, it binds to tubulin impairing intracellular transport mechanisms and interferes with protein synthesis.
Trifluoperazine 2HCl	Neurological Disease	Autophagy	Trifluoperazine is a dopamine D2 receptor inhibitor with IC50 of 1.1 nM.
Triflupromazine HCl	Neurological Disease	Others	
Trifluridine (Viroptic)	Infection	DNA/RNA Synthesis	Trifluridine (Viroptic) is an anti-herpesvirus antiviral drug, used primarily on the eye.
Triflusal	Infection	COX	Triflusal irreversibly inhibits the production of thromboxane-B2 in platelets by acetylating cycloxygenase-1.
Trilostane	Endocrinology	Dehydrogenase	Trilostane is an inhibitor of 3 β-hydroxysteroid dehydrogenase used in the treatment of Cushing's syndrome.
Trimebutine	Neurological Disease	Opioid Receptor	Trimebutine is an agonist of peripheral mu, kappa and delta opiate receptors, used as spasmolytic agent for treatment of both acute and chronic abdominal pain.
Trimethoprim	Infection	Others	Trimethoprim is a bacteriostatic antibiotic mainly used in the prophylaxis and treatment of urinary tract infections.
Trimipramine Maleate	Neurological Disease	Others	
Tripelennamine HCl	Neurological Disease	Histamine Receptor	Tripelennamine is a widely used H1 antagonist, inhibiting PhIP glucuronidation with IC50 of 30 μM.
Trometamol		Others	Trometamol is a proton acceptor used to treat academia.
Tropicamide	Neurological Disease	AChR	Tropicamide is an anticholinergic and a muscarinic receptor subtype M4-preferring antagonist with IC50 of 8.0 nM.
Tropisetron	Neurological Disease	5-HT Receptor	Tropisetron hydrochloride is a selective 5-HT3 receptor antagonist and α7-nicotinic receptor agonist with an IC50 of 70.1 ± 0.9 nM for 5-HT3 receptor.
Trospium chloride (Sanctura)	Cardiovascular Disease	AChR	Trospium chloride (Sanctura) is a competitive muscarinic cholinergic receptor antagonist.

COMPOUND	INDICATION	TARGETS	BRIEF DESCRIPTION
Troxipide	Digestive system disease	Others	Troxipide is a novel systemic non-antisecretory gastric cytoprotective agent with anti-ulcer, anti-inflammatory and mucus secreting properties irrespective of pH of stomach or duodenum.
Tylosin tartrate	Neurological Disease	Others	Tylosin tartrate is a macrolide antibiotic approved for the control of mycoplasmosis in poultry.
Ulipristal	Infection	Estrogen/progestogen Receptor	Ulipristal is a selective SPRM for emergency contraception after an unprotected intercourse or contraceptive failure.
Uracil		Others	Uracil is one of the four nucleobases in the nucleic acid of RNA can be used for drug delivery and as a pharmaceutical.
Urapidil HCl	Respiratory Disease	5-HT Receptor	Urapidil hydrochloride is a hydrochloride salt form of urapidil which is α1-adrenoceptor antagonist and 5-HT1A receptor agonist with pIC50 of 6.13 and 6.4 respectively.
Uridine	Vermifuge	DNA/RNA Synthesis	Uridine is a nucleoside, contains a uracil attached to a ribose ring via a β-N1-glycosidic bond.
Ursodiol (Actigal Urso)	Metabolic Disease	Others	Ursodiol reduces cholesterol absorption and is used to dissolve (cholesterol) gallstones. (IC50=0.22 μM)
Valaciclovir HCl	Infection	Others	Valaciclovir hydrochloride is an antiviral drug used in the management of herpes simplex, herpes zoster, and herpes B.
valganciclovir hydrochloride	Endocrinology	Others	Valganciclovir HCl is a prodrug for ganciclovir with antiviral activity used to treat cytomegalovirus infections.
Valnemulin HCl	Infection	Others	Valnemulin HCl is a broad-spectrum bacteriostatic agent inhibiting protein synthesis in bacteria by binding to the peptidyl transferase component of the 50S subunit of ribosomes.
Valproic acid sodium salt (Sodium valproate)	Cardiovascular Disease	GABA Receptor,HDAC,Autophagy	Valproic acid sodium salt (Sodium valproate) is a HDAC inhibitor with IC50 of 0.4 mM and also inhibits GABA-transaminase or succinic semialdehyde dehydrogenase.
Valsartan (Diovan)	Cardiovascular Disease	RAAS	Valsartan (Diovan) is an angiotensin II receptor antagonist with IC50 of ranging from 39.5 to 116 μM.
Vandetanib (Zactima)	Cancer	VEGFR	Vandetanib is a VEGFR and EGFR antagonist and a tyrosine kinase inhibitor with IC50 of 60, 90, 40 nM for HUVEC proliferation, PC-9 cells and tyrosine kinase activity, respectively.
Vardenafil (Vivanza)	Infection	PDE	Vardenafil(Vivanza) is a new type PDE inhibitor with IC50 of 0.7 and 180 nM for PDE5 and PDE1, respectively.
Vecuronium Bromide	Neurological Disease	Others	Vecuronium (Norcuron) is a muscle relaxant in the category of non-depolarizing blocking agents.
Vemurafenib (PLX4032)	Cancer	Raf	PLX4032 (Vemurafenib, RG7204, Zelboraf, RO5185426) is a novel and potent inhibitor of B-RafV600E with IC50 of 31 nM.

COMPOUND	INDICATION	TARGETS	BRIEF DESCRIPTION
Venlafaxine	Neurological Disease	5-HT Receptor	Venlafaxine (Effexor, Efexor) is an arylalkanolamine serotonin-norepinephrine reuptake inhibitor (SNRI).
Verteporfin (Visudyne)	Endocrinology	VDA	Verteporfin(Visudyne), a benzoporphyrin derivative, is a medication used as a photosensitizer for photodynamic therapy.
Vidarabine (Vira-A)	Infection	DNA/RNA Synthesis	Vidarabine (Vira-A) is an antiviral drug which is active against herpes simplex and varicella zoster viruses.
Vildagliptin (LAF-237)	Metabolic Disease	DPP-4	Vildagliptin (LAF-237) inhibits DPP−4 with IC50 of 2.3 nM.
Vinblastine		Microtubule Associated	Vinblastine inhibits microtubule formation and suppresses nAChR activity with IC50 of 8.9 μM in a cell-free assay, used to treat certain kinds of cancer.
Vincristine	Cancer	Autophagy,Microtubule Associated	Vincristine Sulfate is a microtubule function inhibitor with IC50 of 10.4±1.1, 28.1±3.4, 22.4±2.1 μM for HL-60, Bel7402, HO-8910, respectively.
Vinorelbine Tartrate		Microtubule Associated	Vinorelbine Tartrate is a semi-synthetic vinca alkaloid, and inhibits mitosis through interaction with tubulin.
Vismodegib (GDC-0449)	Cancer	Hedgehog/Smoothened	GDC-0449 (Vismodegib, HhAntag691) is a potent, novel and specific hedgehog inhibitor with IC50 of 3 nM and also inhibits P-gp with IC50 of 3.0 μM.
Vitamin B12	Metabolic Disease	Others	Vitamin b12 is a water soluble vitamin with a key role in the normal functioning of the brain and nervous system, and for the formation of blood.
Vitamin C (Ascorbic acid)	Respiratory Disease	Others	Ascorbic Acid (vitamin C) is a water-soluble vitamin indicated for the prevention and treatment of scurvy.
Vitamin D2	Endocrinology	Others	Vitamin D2 is a selective inhibitors of mammalian DNA polymerase A (pol A) with IC50 of 123 mM.
Vitamin D3 (Cholecalciferol)	Cardiovascular Disease	Others	Vitamin D3 (Cholecalciferol) is a form of vitamin D, binds and activates a H305F/H397Y mutant vitamin D receptor (VDR) with EC50 of 300 nM.
Voglibose	Metabolic Disease	Others	Voglibose is an N-substituted derivative of valiolamine, excellent inhibitory activity against α-glucosidases and its action against hyperglycemia and various disorders caused by hyperglycemia.
Voriconazole	Infection	P450 (e.g. CYP17)	Voriconazole (VFEND) is a triazole antifungal medication that is generally used to treat serious, invasive fungal infections.
Vorinostat (SAHA)	Cancer	HDAC,Autophagy	Vorinostat also known as SAHA, Zolinza, MK-0683 is an HDAC inhibitor. Vorinostat CAS No 149647-78-9 with purity >99% & solubility DMSO is available.
XL-184 (Cabozantinib)	Cancer	Tie-2,TAM Receptor,FLT3,VEGFR,c-Met,c-Kit	XL184 (Cabozantinib) is a potent VEGFR2 inhibitor with IC50 of 0.035 nM and also inhibits c-Met, Ret, Kit, Flt-1/3/4, Tie2, and AXL with IC50 of 1.3 nM, 4 nM, 4.6 nM, 12 nM/11.3 nM/6 nM, 14.3 nM and 7 nM, respectively.
Xylazine HCl	Cardiovascular Disease	Adrenergic Receptor	Xylazine HCl is α2 class of adrenergic receptor agonist.

COMPOUND	INDICATION	TARGETS	BRIEF DESCRIPTION
Xylometazoline HCl	Infection	Adrenergic Receptor	Xylometazoline is an α-adrenoceptor agonist commonly used as nasal decongestant, exhibits highest potency at α2B-adrenoceptor subtype with EC50 of 99 μM. Phase 4.
Xylose	Metabolic Disease	Others	Xylose is a sugar first isolated from wood.
Zafirlukast (Accolate)	Inflammation	Others	Zafirlukast is a leukotriene receptor antagonist (LTRA). (IC50=0.6μM, IC50=7.0 μM for CYP2C9)
Zalcitabine	Infection	Reverse Transcriptase	Zalcitabine is a nucleoside analog HIV reverse transcriptase inhibitor (NARTI).
Zaltoprofen	Inflammation	COX	Zaltoprofen is an inhibitor of Cox-1 and Cox-2 for treatment of arthritis.
Zanamivir		Others	Zanamivir is a neuraminidase inhibitor used in the treatment and prophylaxis of influenza caused by influenza A virus and influenza B virus.
Zidovudine (Retrovir)	Cardiovascular Disease	Reverse Transcriptase	Zidovudine (Retrovir) is a reverse transcriptase inhibitor.
Zileuton	Respiratory Disease	Others	Zileuton (ZYFLO) is an orally active inhibitor of 5-lipoxygenase, and thus inhibits leukotrienes (LTB4, LTC4, LTD4, and LTE4) formation.
Ziprasidone hydrochloride	Neurological Disease	5-HT Receptor,Dopamine Receptor	Ziprasidone was the fifth atypical antipsychotic.
Zolmitriptan (Zomig)	Neurological Disease	5-HT Receptor	Zolmitriptan (Zomig) is a selective serotonin receptor agonist.
Zonisamide	Neurological Disease	Sodium Channel	Zonisamide is a sulfonamide anticonvulsant approved for use as an adjunctive.
Zoxazolamine	Neurological Disease	Others	Zoxazolamine is a centrally acting myorelaxant, which is formerly used as an antispasmodic and uricosuric.